THE EFFECTS OF ENVIRONMENTAL ENRICHMENT ON STRESS-INDUCED EATING DISTURBANCES IN RATS

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Abstract

Eating disorders are serious psychological disorders associated with debilitating lifestyle, multiple health problems and high rates of suicidality and mortality. Despite extensive research, the aetiology of eating disorders still remains unclear. Amongst the identified risk factors for eating disorders, stress has been frequently studied. The purpose of the present study was to explore the possibility that tail-pinch administered to rats could provide an animal model of stress-induced eating disturbances in humans, and whether environmental enrichment might ameliorate the effects of stress. In Experiment 1, we compared eating behaviours of rats that were reared in either enriched or standard environments and later exposed to tail-pinch and allowed to eat when food deprived. The study showed that a single exposure to tail-pinch induced eating disturbances in most of the rats. When rats were not food deprived, but were conditioned to eating when placed in test chamber, tail-pinch suppressed eating in all rats, but significantly more for rats reared under standard than in enriched conditions. Experiment 2 used a between-subjects design in which rats were reared in either a standard or enriched environment, and were either exposed to tail-pinch or not exposed during sessions in which they were not food deprived and allowed to eat. Tail-pinch suppressed the food intake of rats reared in enriched but not standard environments. Although this finding appeared to contradict results of Experiment 1,

analysis of body weight revealed that exposure to tail pinch suppressed increases in weight gain across sessions more for rats reared in standard than enriched environments. The suppression of food intake during test sessions for enriched but not standard rats exposed to tail-pinch was attributed to differences in contextual conditioning and discrimination of the test chamber from home cages. Overall, results of the present study suggest that rats reared in enriched environments were more resilient to the effects of tail-pinch as a stressor. Implications of these findings for the understanding of human eating disorders are discussed.

Chapter 1: Introduction

Eating disorders such as anorexia nervosa and bulimia are debilitating and often have poor prognoses. Although a great deal of research has been dedicated to understanding these disorders, many aspects regarding their aetiology and risk factors still remain unclear. Among the identified risk factors for eating disorders, stress has been extensively studied, but research has often yielded inconsistent results. For example, cross-sectional studies have often showed that stress is significantly correlated with disturbed eating (Leon, Fulkerson, Perry & Cudeck, 1993; Rastam & Gillberg, 1992; Pike, Wilfley, Gairburn, Dohm, & Striegel-Moore, 2006; Wolf & Crowther, 1983), whereas longitudinal studies have often found that stress is unrelated to the development of eating disorders (Leon, Fulkerson, Perry & Cudeck,1993; French, Perry, Leon, G.R. & Fulkerson 1995; Patton et al., 1990). A better understanding of the effects of stress on individual's eating behaviours may lead to the development of successful prevention and treatment of these disorders. The goal of the present research was to contribute to the development of an animal model for stress-induced eating disorders. Specifically, we studied the effects of tail-pinch on rats' consummatory behaviour, and whether these effects were

moderated by rearing in an enriched environment. Our general hypothesis was that environmental enrichment should ameliorate the effects of stress on eating behaviour.

The first part of the present thesis consists of a literature review that covers a number of research areas. It begins with a general introduction to the epidemiology of eating disorders, followed by a review on the risk factors for these disorders. Then we present evidence regarding the relationship between stress and coping in human studies. Next, reasons for studying animal models of eating disorders are outlined, followed by a review of studies on the effects of stress in animals. A range of commonly-used stressors and their effects on animals are described, with a detailed review on the effects of tail-pinch on animals. Lastly, a review of the effects of environmental enrichment on animals is included. The empirical portion of the thesis comprised two experiments, which investigate the effects of tail pinch on eating behaviour in rats reared under enriched or standard laboratory housing conditions. Finally, there is a general discussion, where the findings of the present study are discussed and compared with past findings, and implications considered for understanding human eating disorders.

1.1 Eating Disorders

As the public's awareness of psychological disorders has increased, 'eating disorder' has become a term that has been commonly heard in the popular media. Eating disorders refer to a group of disorders characterized by clinically-significant body image disturbances and abnormal eating behaviours (Hsu, 1990, p1). In the latest revision of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR, American Psychiatry Association, 2005), three disorders have been included as various forms of Eating Disorders: Anorexia Nervosa (AN), Bulimia Nervosa (BN), and Eating Disorder Not Otherwise Specified (EDNOS); while Binge Eating Disorder has been placed in Appendix B as an disorder for further study.

Eating disorders are highly prevalent in Western societies. A recent study conducted by Faravelli et al. (2006) based on an Italian community revealed a prevalence rate of 1.21% for eating disorders. A review (Hoek & van Hoeken, 2003) concluded amongst young females in the Western Europe and the United States, revealed an average prevalence rate of approximately 0.3% for AN, approximately 1% for BN, at least 1% for BED. Wade, Crosby and Martin (2006) examined 1002 female twins in Australia, and found a 1.9% life-time prevalence rate for AN, 2.9% for BN, and an additional 2.9% for BED.

The long-term prognosis for eating disorders is not particularly favourable. Faravelli et al. (2006) found that seven years after the patients were first assessed in

their study, 50% were fully recovered, 26.9% remained affected by an eating disorder, and 23.1% showed some eating disorder symptoms. Similarly, Wade, Crosby and Martin (2006) also suggests that in their study, even though 75% of the participants who was diagnosed with eating disorder at some their life time no longer met the criteria for eating disorders, only a minority of the people appeared to be asymptomatic.

Patients with eating disorders, especially those with AN, are often associated with several serious medical problems, including electrolytes imbalance, gastric disorders, cardiovascular diseases, and renal failure (Hsu, 1990, pp43-47). A number of mental health problems such as affective disorders (Perez, Joiner & Lewinsohn, 2004; Spindler & Milos, 2007), anxiety disorders (Kaye, Bulik, Thornton, Barcarich, & Masters, 2004; Spindler & Milos, 2007; Swinbourne & Touyz, 2007 for a review), substance-related disorders (Bulik et al., 2004; Spindler & Milos, 2007; Wiseman et al., 1999) and personality disorders (Sansone, Levitt & Sansone, 2005; Spindler & Milos, 2007) are also commonly associated with eating disorders. Patients with eating disorders have been found to have an increased risk for engaging in self-injurious behaviours (Pompili, Girardi, Tatarelli, Ruberto & Tatarelli, 2006). Moreover, patients with eating disorders have reported high rates of suicide attempts. In their review, Franko and Keel (2006) found that the rates for patients with AN ranged from

three to 20%, and that for patients with BN ranged from 25 to 35%. With the high comorbidity of physical complications and the high rate of suicidality, patients with AN have been reported as having the highest mortality rate of all psychological disorders (Conwell, 2005; Millar et al., 2005). In a twelve-year follow up study, Fichter, Quadflieg, and Hedlund (2006) found an almost 9-fold risk of mortality in people with AN compared to their counterparts in the general population.

1.2 Risk factors for eating disorders

Despite the extensive research that has been conducted in order to understand the risk factors for eating disorders, important aspects remain poorly understood (Striegel-Moore & Bulik, 2007). Risk factors proposed to be associated with eating disorders range from cultural, psychological, to physiological ones. The most well researched cultural factor is the internalisation of the thinness ideal, which has been linked with disrupted eating patterns (Bulik et al., 2006; Hoek & Van Hoeken, 2003). Biological factors such as serotonergic imbalance (Audenaert et al., 2003; Kaye et al., 2005; Wolfe, Metzger & Jimerson, 1997) and diet restraint (Herman & Polivy, 1984; Patton & King, 1991) have also been well documented. Many studies had also dedicated in the understanding of psychological risk factors for eating disorders, and these factors include perfectionism (Eckerd, 2005; Frost, Marten, Lahart &

Rosenblate, 1990; Halmi et al., 2000), low self-esteem (Chen, Fu, Chen & Wang, 2007; Granillo, Jones-rodriguez & Carvajal, 2005; Vohs, Bardone, Joiner Jr., Abramson & Heatherton, 1999), body dissatisfaction (Ackard & Peterson, 2001; Phelps, Johnston & Augustyniak, 1999; Polivy & Herman, 2002; Striegel-Moore, Wilson, Wilfley, Elder & Brownell, 1998; Telch & Stice, 1998), impulsivity (Matsunaga et al., 2000; Wolfe, Jimerson & Levine, 1994), and poor coping ability (Henderson & Huon, 2002; Shatford & Evans, 1986).

1.2.1 Stress and coping in humans

Stress has been shown to be a risk factor for many psychological disorders including depression (Kendler et al., 1995; Kendler, Karkowski & Prescott, 1999) and anxiety disorders (Pine, Cohen, Johnson & Brook, 2002). Many studies have also been conducted in order to identify such relationship between stress and eating disturbances. In a review of research on emotions and eating, Macht (2008) reported that most individuals experience some change in eating patterns when experiencing stress, with averages of 30% and 48% showing either an increase or decrease in food intake, respectively. Different approaches have been taken in order to examine the construct of stress, and one of the most common approaches is to focus on the role of adverse life events in individuals with eating disorders. Life events that are commonly

considered in these studies include major changes in circumstances (Horesh et al., 1995; Margo, 1985), events of loss (Lacey, Coker & Birtchnell, 1986), threats to physical safety (Welch, Doll & Fairburn, 1997), and sexual conflicts (Lacey, Coker & Birtchnell, 1986). Compared to healthy control, significantly more adverse life events were reported by individuals with eating disorders in many studies (Rastam & Gillberg, 1992; Pike et al., 2006; Welch, Doll, & Fairburn, 1997). However, studies looking at adverse life events alone do not take individual differences, such as temperament and physiological stress response, into consideration, despite their mediating effects on the perception of adverse life events (Koo-Loeb, Costello, Light & Girdler, 2000; Pryor & Wiederman, 1996). Many studies have found a significant correlation between perceived stress levels and eating disturbance (Crowther & Chernyk, 1986; Soukup, Beiler & Terrell, 1990; Striegel-Moore, Silberstein, Frensch & Rodin, 1989; Wolf & Crowther, 1983; Leon, Fulkerson, Perry & Cudeck, 1993). However, these studies were all cross-sectional in nature, whereas most longitudinal studies have yielded somewhat different findings. Patton and colleagues' (1990) found a positive correlation between perceived stress level and eating disturbance in the cross-sectional part of their study, whereas their 12-month follow up did not show the same correlation. Similar findings were also observed in Leon, French and colleagues (1993; 1995). Despite this, Streigel-Moore et al. (2007) showed a positive

longitudinal relationship between perceived stress level and eating disturbances, and suggested that perceived stress may be antecedent to the development of binge eating disorder and BN.

Taken together, individuals with eating disorders appear to have a higher stress level than healthy individuals. This is consistent with the high co-morbid rate between eating disorders and anxiety disorders (see Swinbourne & Touyz, 2007, for review). Specifically, it was noted that most individuals with this comorbidity suffered from anxiety disorder prior to the onset of eating disorders (Bloks, Spinhoven, Callewaert, Willemse-Koning & Turksma, 2001; Bulik, Sullivan & Kendler, 2003; Godart et al., 2003). Although it is tempting to conclude that the stress has a catalyst role in the development of eating disorder, many found that the stressors reported by patients with eating disorders were usually similar to those that were experienced by individuals with other types of psychological disorders, or psychologically healthy individuals (Ball & Lee, 2000). This finding led many to speculate that the different manifestations of the stressful experience depend on the individuals' repertoire of coping strategies (Ball & Lee, 2000). Folkman (1984) suggested that the initiation of an individual's coping behaviour is dependent on the individual's perception of the effect of stress, and the appraisal of one's ability to cope with it. Furthermore, individuals with inadequate problem-solving skills would

be less able to cope effectively with stress, and eating disturbance may be one of the expressions of an ineffective coping style (Folkman & Lazarus, 1984; Troop, Holbrey, Trowler & Treasure, 1994). Unsurprisingly, studies have found that individuals with eating disorders were more avoidant and passive in response to problems (Bloks et al., 2001; Yager, Rorty & Rossotto, 1995), adopt more maladaptive coping strategies (Aime, Sabourin & Ratte, 2006; Koff & Sangani, 1997), and had fewer use of social diversion (Hendley, 2002). In their study, Troop and Treasure (1997) found that individuals with bulimic symptoms tended to adopt ruminating coping styles, whereas individuals with anorexic symptoms tended to adopt cognitive avoidance. Both of these coping styles were found to be less frequently used in normal controls.

Nevertheless, the catalyst role of tress and maladaptive coping on the onset of eating disturbance has only been found in cross-sectional studies but not in longitudinal studies. In their study, Ball and Lee (2002) found a strong short-term relationship amongst stress and maladaptive coping and disordered eating, but when examined over a six-month period, the relationship was weak. Similarly, another study (Patton et al., 1990) found no significant relationship over a 12-month period. Taken together with the findings of the cross-sectional studies, it is suggested that the relationship between stress and maladaptive coping and disordered eating is strong yet short-lived (Ball & Lee, 2002). Furthermore, it is also likely that stress and

maladaptive coping may have induced third variables, such as weight dissatisfaction (Ball & Lee, 2002), that have a stronger longitudinal relationship with disordered eating, and eventually lead to eating pathology.

1.2.2 Rationale for using animal models

Studies using animal models hold the premise that animals are, to some degree, similar to humans because of shared evolutionary history (Overmier & Carroll, 2001, p4). With animal studies, experimental procedures that cannot be performed on humans due to ethical or legal reasons may be permissible. As a result, animal studies have yielded a wealth of knowledge in human medical science, and more recently, human psychology (Koolhaas, de Boer, de Ruiter & Bohus, 1999, pp.3-4). Nevertheless, the use of animal models in psychology has been controversial for reputed over-simplicity (Shapiro, 1998, p149) and overemphasis on the direct relevance to humans (Koolhaas, de Boer, de Ruiter & Bohus, 1999, p259). In the case of eating disorders, BN and obesity are often conceptualised as "excessive food intake", and AN as "inadequate food intake" (Shapiro, 1998, p149). Although these conceptualisations make animal models feasible, they fail to capture some aspects of these disorders. Psychological disorders are highly complex in their causes and maintenance, involving multi-levels of factors such as physiology, cognition,

individual disposition, interpersonal relationships, environment, culture, and so on (Shapiro, 1998, p117). Nonetheless, even though one cannot examine the complex interplays of all the factors of a certain psychological disorder through animal models, they enable the discovery of basic laws of behaviour (for instance, Pavlov's classical conditioning, 1927), which are also applicable to humans and have served prominently in the development of effective therapeutic interventions. For example, Lewinsohn's (1972) behavioural theory was based on Skinner's (1948) principles of operant conditioning, and has been applied extensively in the cognitive behavioural therapy in a number of psychological disorders such as depression)

1.2.3 Stressors used in animals

Many studies have focused on attempts to induce the equivalent of human emotional experiences in animals, in particular, the experience of stress has been extensively studied. Stress is often induced through stimulating the animal, or by placing the animal in a situation that provokes fear and/or anxiety. A number of commonly used procedures are described below.

Inescapable electric shock has been one of the most commonly used methods to induce stress in animals (Corwin & Buda-Levin, 2004). The physiological effects of electric shocks have been well documented, and include increased food intake (Hagan

et al., 2002; Hagan, Chandler, Wauford, Rybak & Oswald, 2003; Ullman, 1951) without increased total body weight (Sterritt & Shemberg, 1963), reduced food intake (Richards, Job & Boakes, 1997; Job & Barnes, 1995), and body weight loss (Brennan, Job, Watkins & Maier,1992; Dess, 1991; Richards, Job & Boakes, 1997) even without food reduction (Dess, Chapman & Minor, 1988).

Social isolation is also used as a stressor, and animals subjected to this procedure have displayed increased food intake (Miller, Mirsky, Caul & Sakata, 1969; Shelley, 1965), increased sucrose fluid intake (Hall, Huang, Fong, Pert & Linnoila, 1998), increased body weight (Morgan, 1973; Shelley, 1965), although sometimes body weight has not changed (Rosen, 1961), and changes in food intake have not been consistent (Weltman, Sackler, Sparber & Opert, 1962). In addition, even though social isolation is stressful for animals like monkeys, rats and mice, for some other animals such as Syrian hamsters, group housing is more stressful than individual housing (Meisel, Hays, Del Paine, & Luttrell, 1990).

Other stressors have also been used to study their effects on animals' eating behaviour. Social defeat was induced by placing rats in the cage of an aggressive male conspecific for a fixed time period (e.g., one hour). Rats that experienced a single social defeat showed body weight reduce but no food intake change, and those experienced it twice showed reduction in both body weight and food intake (Meerlo,

Overkamp, Daan, van den Hoofdakker & Koolhaas, 1996). When subjected to stressful noise, one study showed that only one third of rats showed reduced food intake (Macht, Krebs, Weyers & Janke, 2001), and another showed reduced eating duration and increased eating speed and latency to begin eating (Krebs, Macht, Weyers, Weijers & Janke, 1996). Increased food intake in response to stressful noise has been reported (Rasbury & Shemberg, 1971; Wilson & Cantor, 1986). Female rats exposed to maternal separation showed increased eating (Iwasaki, Inoue, Kiriike & Hikiji, 2000) as well as less body weight but increased consumption in more palatable food (McIntosh, Anisman & Merali, 1999). Furthermore, limited living space was found to be associated with increased food intake in rats after restricted feeding (Inoue et al., 1998), and cold water swim was associated with more high fat food consumption in rats (Vaswani, Tejwana & Mousa, 1983).

Amongst all stressors, tail-pinch has been perhaps the most commonly-used method to provide an animal model of stress-related eating disturbances. The effects of tail-pinch on eating behaviour were first observed by Antelman and colleagues (1975), who reported that rats that were not food deprived responded to tail-pinch by eating, licking or gnawing food that was available. This finding has soon led to the use of tail-pinch in studies looking at effects of medications (Boutelle, Svensson, & Fillenz, 1990; Czech, Klosterman & Sueur, 1998; Hawkins, Fuller, Baumeister &

McCallum, 1994; Mittleman, Rushing & Winders, 1993; Morley & Levine, 1981; Levine & Morley, 1981) and brain damage (Greenspon & Fass, 1981; Rowland, Marques & Fisher, 1980) on tail-pinch-induced hyperphagia, with the assumption that tail-pinch would inevitably increase eating (Greeno & Wing, 1994).

However, research on the behavioural effects of tail pinch, in the absence of other physiological or pharmacological interventions, has been limited. A literature search conducted for this thesis identified only five studies that included details of non-tail-pinched rodents that were not brain damaged or medicated to compare with their tail-pinched counterparts (Levine & Morley, 1981; Meadows, Phillips & Davey, 1988; Murphy, Porter, & Heath, 1985; Robbins, Phillips & Sahakian, 1977;Rowland & Antelman, 1976). Table 1 shows the details of the studies. The following are descriptions of the summaries of the six studies. For the purpose of the present literature review, only the aspects that are relevant to tail-pinch-induced food consumption of intact animals are included.

In Rowland and Antelman's (1976) study, six surgically-intact female rats were included in an experiment looking at effects of tail-pinch. The rats were assigned to control and tail-pinch groups, and their consumption of sweetened milk was recorded over six 10- to15- minute daily eating sessions for five consecutive days. The rats were not food deprived, and did not undergo eating training to establish eating

behaviours prior to the testing sessions. The study showed that when subjected to tail-pinch, rats immediately showed dramatic hyperphagia, which resulted in higher daily weight increases and higher total body weights.

Robbins, Phillips and Sahakian's (1977) study included four medication-free male rats, each of which underwent a one-minute control session immediately before the one-minute tail-pinch session, with a total of two tail-pinch sessions a day, for two consecutive days. The rats were not food deprived, and eating behaviour was not established prior to the testing sessions. The within-group comparisons of the four rats showed that tail-pinch significantly increased the duration of eating.

Levine and Morley (1981) studied the effect of tail-pinch on 10 male rats, with six rats assigned to the control group and four rats to the tail-pinch group. The testing sessions consisted of three 5-minute trials spaced 20 minutes apart, which were conducted four times daily for 10 days. The rats were not food deprived, and the eating behaviour was not established prior to the testing sessions. One of the four rats showed no tail-pinch-induced behaviour after the first 24 hours. The spontaneous food intake in the other three rats reduced when first subjected to tail-pinch, and returned to the level similar to that prior to tail-pinch. The tail-pinched rats also showed an overall decrease in weight gain compared to the controls.

Murphy, Porter and Heath's (1985) study included the effects of tail-pinch on eating in male rats. In the initial tail-pinch screening, only 12 out of 20 rats displayed increased eating when subjected to tail pinch. Later in the study, six rats were assigned to the control group and 5 to the tail-pinch group, and all rats were food restricted to maintain 80% of their free-feeding body weight before the testing sessions. During the baseline, the rats were trained to press a lever for food pellets with a fixed-interval (FI) one-minute reinforcement schedule. The experimental procedure included three phases. In Phase 1, the rats were tested in their assigned conditions. Phase 2 involved a reverse of conditions, where tail-pinch rats were not tail-pinched, and control rats were tail-pinched. Phase 3 consisted of a replication of Phase 1. Each session lasted 30 minutes. The study showed that the rats displayed more eating in the tail-pinch sessions compared to the non-tail-pinch sessions.

Meadows, Phillips and Davey (1988) examined the effects of tail-pinch on consumption of palatable liquid food by rats and hamsters. Eight male rats and eight male hamsters were both equally assigned to control and tail-pinch conditions. The rats were not food deprived and did not undergo eating training to establish eating behaviours prior to the testing sessions. The experiment consisted of three phases, the baseline phase, the tail-pinch phase, and the return-to-baseline phase. Six 10-minute sessions were conducted daily for six days. The study showed that when subjected to

tail-pinch, rats displayed an overall increase of food consumption, whereas hamsters showed no overall increase in food consumption, but an increased rate of consumption.

Thus the literature review found that tail-pinch did not inevitably increase food intake, rather, some studies even showed decreased eating behaviours. The rats' response to tail-pinch did not appear to depend systematically on procedural differences. Overall, tail-pinch did appear to affect eating behaviours, but the direction of the effect varied across studies. It is important to note that all the studies mentioned here were based on relatively small sample sizes, which made individual differences in their tail-pinch reactivity amongst the animals more significant. The current literature review also showed that other than tail-pinch, most of the manipulations commonly used for inducing stress in animals had inconsistent effects on eating behaviours. This suggests that eating in animals involves a complicated mechanism that may be moderated by multiple factors such as individual differences or environmental variables. The goal of the present study was to examine further the effects of tail-pinch on rats' consummatory behaviour, and specifically to explore the possible impact of rearing in an enriched environment on these effects.

Table 1: Tail-pinch studies included un-medicated and surgically intact animals

	1	2	3	4	5
Study	Rowland & Antelman (1976)	Robbins, Phillips & Sahakian (1977)	Levine & Morley (1981)	Murphy, Porter & Heath (1985)	Meadows, Phillips & Davey (1988)
Species	Rats	Rats	Rats	Rats	Rats and Hamsters
Strain	Sprague-Dawley rats	Hooded rats (not specified)	Sprague-Dawley and Fisher rats	Albino Sprague-Dawley	Hooded Lister rats & Golden hamsters
Sex	Female	Male	Male	Male	Male
Age	Adult	50 days of age	Between 150 and 300 g	Averagely weighted 367.3g	Adult
No. of subjects	24 (6 surgically intact, 10 ovariectomized, and 8 ovariectomized and injected daily with hormone replacement), with 15 rats in non-TP group and 9 rats in TP	36	10 (4 TP rats and 6 controls)	11 (6 control, 5 TP)	8 rats and 8 hamsters (4 rats and 4 hamsters in TP group, 4 rats and 4 hamsters in control group)

	group.				
Food type	Sweetened milk	Food pellet	Purina lab chow pellet	Noyes food pellet	Palatable liquid food solution
Food deprivation	No food deprivation	No food deprivation	No food deprivation	80% of the body weight	No food deprivation
Experimental design	6 daily pinch sessions (10- 15 minutes each), spaced at equal interval throughout the 24-hour cycle for 5 consecutive days	Two trials each lasted 60 seconds were separated by 30 minutes on each of 2 consecutive days. Each trial preceded by one minute control trial without TP.	three 5-minute trials 20 minutes apart, repeated four times a day for 10 days.	**Phase 1**: Ten 30-minute TP sessions **Phase 2**: Testing conditions reversed (6 sessions for control, and 8 sessions for TP) **Phase 3**: returning of conditions (6 sessions for the TP and 8 sessions for the control)	Mixed design. 3 phases (10 minute) in a session (ABA), with 6 sessions a day for 6 days.
Result	All TP rats became	TP rats showed more eating	One rat developed	Subjects ate sig. more	**Rats** had a sig. increase

	hyperphagic and obese		tolerance to TP after the first 24 hours and no longer displayed TP behaviours. Three rats showed similar total food intake (spontaneous food intake decreased to half compared to the baseline, and the forced food intake was just over half of the spontaneous food intake in baseline). A decrease in	during the TP phage.	in food intake; **Hamsters** showed NO sig. increase in overall consumption, but a sig. increase in the rate of consumption.

			weight gain compared to the controls. Feed efficiency was reduced during TP.		
Note		Animals administered with chlordiazepoxide of different dosages (0, 5, 10, 20 mg/kg)	All subjects were hypophysectomized (excised of pituitary gland).	12 out of20 showed increased food intake in initial TP and remained in study.	

1.3 Environmental Enrichment

Environmental enrichment has been frequently used in studies to examine the effect that environmental stimulation has on animals. In these studies, environmental enrichment typically refers to the presence of objects that provide distractions for the animals in an otherwise monotonous environment (Belz, Kennell, Czambel & Rubin, 2003). An enriched environment could be created through providing social and/or physical stimuli, which gives the animals more opportunities to play or to perform species-specific behaviours. In contrast, standard environments are those where only the most basic components of a living environment are available (van der Harst, Baars & Spruijt, 2003), such as the ground, the walls, the food and water supply. Research on environmental enrichment originated in the early work of Hebb (1949), as well as Rosenzweig, Krech and Bennett (1956 & 1958), who found that environmental experiences induce changes in animal brains. Rosenzweig and Bennett (1996) suggested that the growth of the brain is highly dependent on the experiences that an animal has, and that sufficient external stimulation enables the full development of the species-specific brain characteristics, and enables the organism to reach its full behavioural potential.

According to the literature, environmental enrichment has generally been found to have a positive effect on animals' physical, cognitive and emotional functioning (Fox , Merali & Harrison, 2006). Environmental enrichment has been associated with increased brain weight and size (Rosenzweig & Bennett, 1969), increased protein synthesis and amount of protein in the cortex (Bennett, 1976), and increased amount (Bennett, 1976) and expression of RNA (Grouse, Schrier, Bennett, Rosenzweig & Nelson, 1978). More food intake and body weight were also found in enriched animals (Roy, Belzung, Delarue, & Chapillon, 2001; van der Weerd et al., 2002),

although Beattie, Walker and Sneddon (1995) and Dudink, Simonse, Marks, de Jonge, and Spruijt (2006) did not find significant difference between enriched and standard pigs. Vinke et al. (2004) raised minks in three conditions differing in the level of enrichment, and observed more variable behaviours in the most enriched minks compared to the most standard minks. The variable behaviours were thought to be elicited by the stimulating elements in the enriched environment. Short term (one week) exposure of enriched environment is associated with more exploratory behaviour and a better and faster learning performance in chickens, although no differences in memory between the enriched and standard chickens was found (Krause, Naguib, Trillmich & Schrader, 2006). It is also associated with better long term memory in pigs (de Jong et al., 2000), better spatial learning, more efficient spatial information processing, and increased ability to adopt different strategies to solve problems in rats (Leggio et al., 2005).

Furthermore, environmental enrichment has been shown to improve memory in animals with brain lesions (Kolb & Gibb, 1991; Will, Rosenzweig, Bennett, Hebert & Morimoto, 1977), prenatal alcohol exposure (Rema & Ebner, 1999), and in old age (Mohammed et al., 1993; Winocur, 1998). Environmental enrichment was also found to induce neurogenesis in rat strains that are known as poor learners and improve their learning (Kempermann, Brandon & Gage, 1998).

Apart from these differences observed between animals reared in enriched and standard environments, researchers have explored whether environmental enrichment might have a protective effect against stressors. Research examining stress-induced cognitive impairment has yielded some interesting findings. For example, Wright and Conrad (2008) reported that after exposure to chronic stress of food restraint, rats reared in a standard environment were found to have impaired spatial memory and

learning, whereas those reared in an enriched environment showed intact spatial memory and learning. Physical differences were also found between enriched and standard animals. Environmental enrichment was associated with a reduced severity of stress-induced gastric ulcer (Rockman, Borowski & Glavin,1986), normal level of hypothalamus-pituitary-adrenal (HPA) response to stressful experiences (Moncek, Duncko, Johansson & Jezova, 2004), and an enhanced natural killer-cell activity, which destroy external agents in the body, thus representing a stronger immune system (Benaroya-Milshtein et al., 2004). Furthermore, studies found that similar cerebral changes were observed in rats exposed to enriched environment in their juvenile (Zolman & Morimoto, 1962), young adulthood (Bennett, Diamond, Krech, & Rosenzweig, 1964; Rosenzweig, Krech & Bennett, 1963) and adulthood (Riege, 1971). These findings indicate that environmental enrichment has effects on animals not only in early stages of life, but also in later stages.

Protective effects have also been found in behavioural studies. Researchers noticed that when being handled by experimenters, enriched mice appeared more at ease, easier to handle, showed fewer movements and maintained a normal breathing rate (Chapillon, Manneche, Belzung & Caston, 1999; Engellenner, Goodlett, Burright, & Donovick, 1982; Pham et al., 1999; Van de Weerd et al., 2002). In open field tests, enriched animals produced less faecal boli (Larsson, Winblad & Mohammed, 2002), showed less locomotion and more interaction with objects placed in the open field arena, and demonstrated faster habituation in novel situations (Engellenner, Goodlett, Burright, & Donovick, 1982; Van de Weerd et al., 2002). De Jong et al. (2000) reported that when exposed to novel environments, enriched piglets had shorter latencies to leave the pen, spent significantly less time in the pen, and spent less time with their mates. These findings suggest that enriched piglets were less fearful and

had less need for social support in novel situations. Similar results were reported by Roy and colleagues' (2001) study of mice using elevated plus maze and open field tests.

Joseph and Gallagher (1980) suggested that a limited behavioural repertoire is developed in animals reared in a standard environment, therefore they tend to over-respond in a testing environment. Furthermore, when compared to enriched rats, it was shown that standard rats displayed more anticipatory behaviour by responding more to a stimulus that was conditioned with sucrose reward (Van der Harst, Baars, & Spruijt, 2003). This indicated that standard rats were more sensitive to positive stimuli than enriched rats. Given that standard environment is considered to be more stressful for rats, the study finding is consistent with studies showing that stress increases sensitivity for rewards and aversive stimuli (Piazza, Deminiere, Le Moal & Simon, 1990).

In addition, a higher level of grooming behaviour has been observed in enriched rats in a novel environment (Brenes Saenz, Villagra & Trias, 2006). Grooming behaviour is known to be a de-arousal mechanism for rats, which may enable them to maintain a lower anxiety level. Brenes Saenz et al. concluded that while enriched rats maintained a low anxiety level with an activated arousal-inhibition system, rats without environmental enrichment appeared to require more time to regulate their anxiety level this way. Furthermore, an enriched environment provides animals with more opportunity to perform species-specific behaviours, and may give them an increased sense of controllability, which reduces negative affects induced by stressors (Van de Weerd, 2002; Wiepkema & Koolhaas, 1993). This is consistent with Bandura's (1977 & 1982) concept of self-efficacy, which refers to individuals' belief of their ability to "produce and regulate events in their lives" (Bandura, 1982, p122).

Individuals with high self-efficacy are thought to be able to maintain a lower level of anxiety through an enhanced sense of controllability (Weems & Silverman, 2006). Moreover, being able to adapt to repeated stress faster may have survival value, as it prepares the animal to cope with environmental demands (Fox, Merali, & Harrison, 2006).

In addition to its protective effects, environmental enrichment is also able to reverse or compensate for negative effects of stressors in animals (Escorihuela, Tobena & Fernandez-Teruel, 1994; Francis, Diorio, Plotsky& Meaney, 2002). This observation is consistent with the findings that environmental enrichment is beneficial not only during early developmental stages, but also in later stages.

Although there have been no studies on environmental enrichment with humans that are directly analogous to those with animals, results from studies regarding effects of other types of environmental factors on stress-coping ability have been done. The studies included negative environmental factors such as childhood abuse and neglect (Futa, Nash, Hansen & Garbin, 2003; Kennedy, Ip, Samra & Gorzalka, 2007; Lieberman & Knorr, 2007), unsupportive family climate (Foss & Holen, 2006; Minuchin, Rosman, & Baker, 1978), poor interpersonal relationship (Foss & Holen, 2006), and accidental traumatic events (Lieberman & Knorr, 2007). The findings showed that chronic exposures to these factors often result in development of an inferior stress coping ability in people (Futa, Nash, Hansen & Garbin, 2003; Lieberman & Knorr, 2007), suggesting the significance of environmental conditions on human behaviours. In addition, negative environmental factors usually elicit negative emotions, which are known to influence subsequent behaviours. For example, Bower (1981) reported that individuals who chronically have negative emotions tended to perceive things in a more negative way.

As for animals, those reared in a monotonous standard environment might be expected to have more negative emotions, including stress, than those reared in an enriched environment. Thus, studies of the effects of environmental enrichment on stress coping ability in non-humans may contribute to the understanding of the effects of the environment to stress-coping in humans.

Although the above-mentioned positive effects of environmental enrichment on animals are well established in the literature, no study has been conducted which jointly examines the effects of environmental enrichment and stress on animals' eating behaviour. Understanding the relationship between environmental enrichment, stressors and consummatory behaviour should enable us to obtain a more complete picture of the protective effects that environmental enrichment provides when animals are exposed to stressors, and hopefully provide insights that are relevant for human behaviour.

Based on the present literature review, we assumed that tail-pinch would induce stress-like experience in rats, and that rats reared in enriched environment would be more resilient to the effects of stress than those reared in a standard environment. The aim of our study was to examine the effects of environmental enrichment in eating behaviours in rats under stress, and we hypothesised that rats reared in enriched environment would show less eating changes caused by tail-pinch than those reared under standard laboratory conditions.

Chapter 2: Experiment 1

Experiment 1 tested the effects of tail pinch on rats' eating behaviour and the possible moderating role of rearing conditions using a mixed within- and between-subjects design. Rats reared under standard and enriched conditions were food-deprived and allowed to eat in an experimental chamber during daily test sessions. After sufficient sessions had been conducted so that food intake was stable, a single test session was conducted in which rats were exposed to tail pinch (acute tail-pinch test). Food deprivation was then discontinued, while rats continued to receive daily sessions in which they could eat in the test chamber. When eating behaviour was again stable, rats were exposed to tail pinch over nine successive sessions (chronic tail-pinch test). In this way, we planned to examine the effects of tail pinch on eating behaviour for rats reared in standard and enriched environments, under conditions in which they were food deprived or not deprived. We also conducted open-field and novel object tests to examine whether there were other behavioural differences between the rats reared under standard and enriched conditions.

2.1 Methods

2.1.1 *Subjects*

The subjects were 16 PVG/C Male Hooded rats. They were housed in a vivarium with a 12:12 hour light/dark cycle, with lights on at 8:00 and off at 20:00, a room temperature of 21/22 degree Celsius, and the humidity at 50% during all segments of the study. After weaning, half of the rats were assigned to the standard condition, whilst the other half to the enriched condition. The rats were housed in groups of four, with food and water *ad libitum* until the experimental phase.

2.1.2 Apparatus

Eight standard cages (29x 23x 44.5 cm) and four enriched cages (49x 43x 64.5 cm) equipped with water and food supplies were used. In each condition, two cages were used as the home cages, and two other cages were used as the replacements when the other two home cages were sent for cleaning. Various toys such as small ladders and tubes were provided in the two enriched cages, and they were replaced every four days. Two standard cages were used for moving the rats to the testing room and for holding the rats prior to testing. The two remaining standard cages were used as the testing cages for the eating tests.

Two metal trays (6cm X 1.5cm X 4cm) containing crushed food chow were used during the experimental sessions. The food trays were placed in two glass containers (15cm X 10cm X 7cm) for gathering food crumbs during the sessions.

Two small paper clips (3cm x 1cm x 0.5cm) were used during the tail-pinch phase. In order to protect the tails of the rats, each paper clip was padded with a small foam pad at the surface of the clip where it clipped on and touched the tails. Two strings (approximately 20cm in length) were attached to the end of the paper clips for controlling the position of the tails. The rats and food trays were weighed both before and after the experimental sessions.

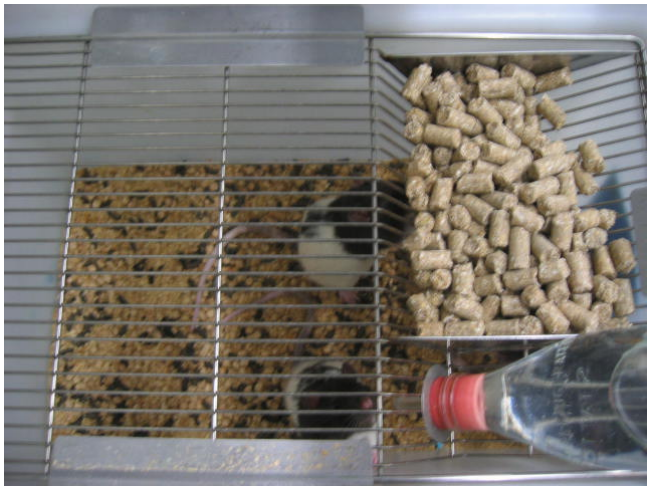

Photo 1: A Standard cage

Photo 2: The enriched cages

An open-field arena (32.5x 61x 61.5 com) was used during the open-field tests. The arena consisted of a wooden floor enclosed by four walls painted in black.
A plastic drinking bottle (20cm X 8cm X 8cm) filled with sand was used as a novel object for the open-field tests.

2.1.3 Procedure

2.1.3.1 OFT

Possible behavioural differences of rats in the two conditions were assessed through two open field tests in Experiment 1. The first open-field test was conducted 30 days after the rats' exposure to the standard and enriched environments over a period of two days. For each open field session, a rat was placed in the peripheral part of the open-field arena. The numbers of times the rat crossed the grid line on the floor and showed rearing behaviour were recorded for six minutes. After the first six minutes, a novel object was placed in the centre the arena for six minutes, and the rat's latency to approaching the novel object was recorded. After the latency period, the time that the rat spent contacting the object, and the number of faecal boli produced by the rat were also recorded, and the rat was removed from the arena. The arena was cleaned between sessions.

The second open-field test was conducted between the 10^{th} and the 11^{th} eating sessions of the first eating test. The procedure for the open-field test was same as that of the first open-field test, except that the latency to contact the novel object and the time spent contacting the object were not recorded.

2.1.3.2 Acute tail-pinch Test

Baseline sessions

Two days after the first open-field test, the rats were trained to eat in the test chamber. Baseline sessions were conducted once a day for 10 days. The rats were deprived of food 18 hours prior to each eating session, and the tests started at 1000 hr everyday. Each rat was placed in the empty test chamber for two minutes for habituation. Then, the food tray in a glass container (containing crushed food chow) was placed in front of the rat for another 10 minutes. After the 10 minutes, the rat was removed from the chamber. The rat and the food tray were weighed both before and after the eating session.

Tail-pinch session

After the baseline phase, the rats experienced one tail-pinch session. The procedure for the tail-pinch session was the same as that for the baseline eating session, except that before the rat entered the testing chamber, a mild tail pinch was applied by placing a paper clip on the rat's tail (approximately 3cm from the tip) which remained throughout the eating session. During the testing, the rat's tail was kept away from the rat's sight by the experimenter holding the string attached to the clip, so that the rat would not attempt to release itself from the clip. After the test, the rat's tail was released by the experimenter. The procedure was repeated after all the rats were assessed.

Post-tail-pinch Baseline Sessions

Two days of baseline eating sessions were conducted after the first tail pinch session. The procedure for the eating sessions was the same as described above.

2.1.3.3 Chronic Tail-Pinch Test

Four days after the first consumption test, the rats underwent the second consumption test, which consisted of 21 daily baseline sessions and nine days of tail-pinch test sessions. The procedure for the second consumption test was different from the first in two ways: The rats were not food deprived prior to the sessions, and instead of having one tail-pinch session followed by two post-tail-pinch baseline sessions, rats were given nine consecutive tail-pinch sessions. Apart from these two differences, the procedure for the second consumption test was the same as that for the first one.

One rat in the standard group showed aggressive behaviour towards the experimenter on the 5th tail-pinch session onward, and was unable to be captured for tail-pinch for the following sessions, thus was excluded from the experiment.

2.2 Results

2.2.1 Acute Tail-pinch Test

2.2.1.1. Food Intake

Average Food Intake in Baseline

Figure 1 shows the average food intake (grams/session) for Group Standard and Group Enriched in the baseline sessions. The right-most data point for both groups represents the average food intake across the last five baseline sessions. For both groups, food intake increased over baseline training and appeared to reach stable levels after about 10 sessions. A repeated-measures analysis of variance (ANOVA) found a significant effect of time, $F(17,238)= 49.33$, $p<.001$, showing that food intake increased across the baseline phase. The main effect of group was not significant ($p > .10$), but the time x group interaction was significant, $F(17,238)= 2.38$, $p<.005$. The

interaction indicates that whereas food intake was greater for Group Enriched during the first few sessions, food intake was similar for both groups at the end of baseline training.

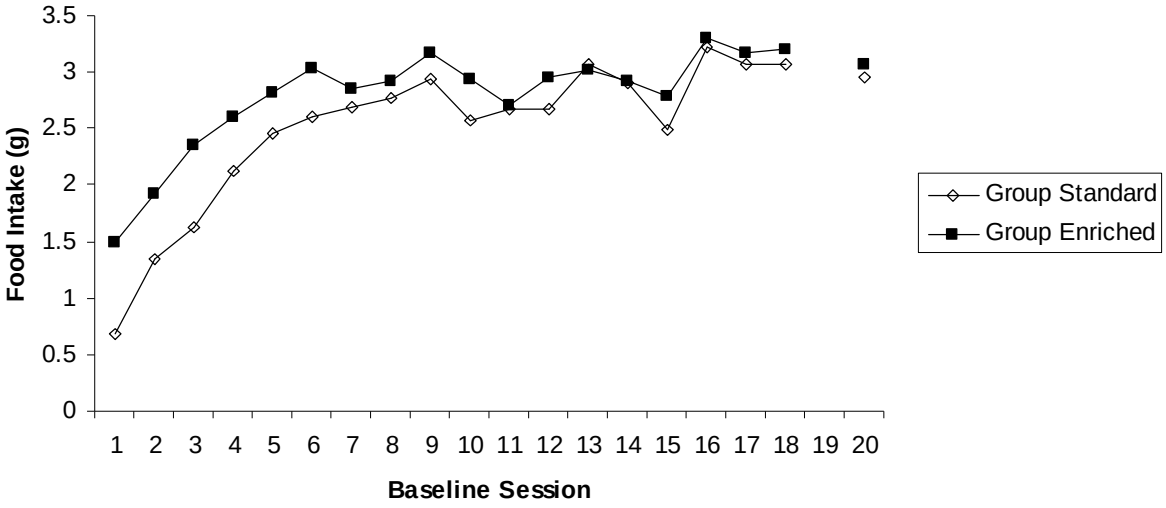

Figure 1. Average food intake of Group Standard and Group Enriched during baseline phase

Food Intake in Baseline, Tail-Pinch Session, and Post-Tail-Pinch Sessions

Figure 2 shows the average amount of food intake of Group Standard and Group enriched during the acute tail-pinch test. Average food intakes during the last two sessions of baseline, and that during the post-tail-pinch sessions are also shown. A repeated measure ANOVA found a significant effect of phase, $F(2,28)=4.64$, $p<.05$. Post-hoc comparisons (Tukey HSD) indicated that food intake was greater during the post-tail pinch sessions than the pre-tail pinch sessions, but there was no significant difference between the tail pinch session and the pre- and post-tail pinch sessions. The analysis found no significant effects of group, or phase x group interaction (both

‘’s> .1). This suggests that there was no systematic difference in food intake when rats were food deprived between rats reared under standard and enriched conditions.

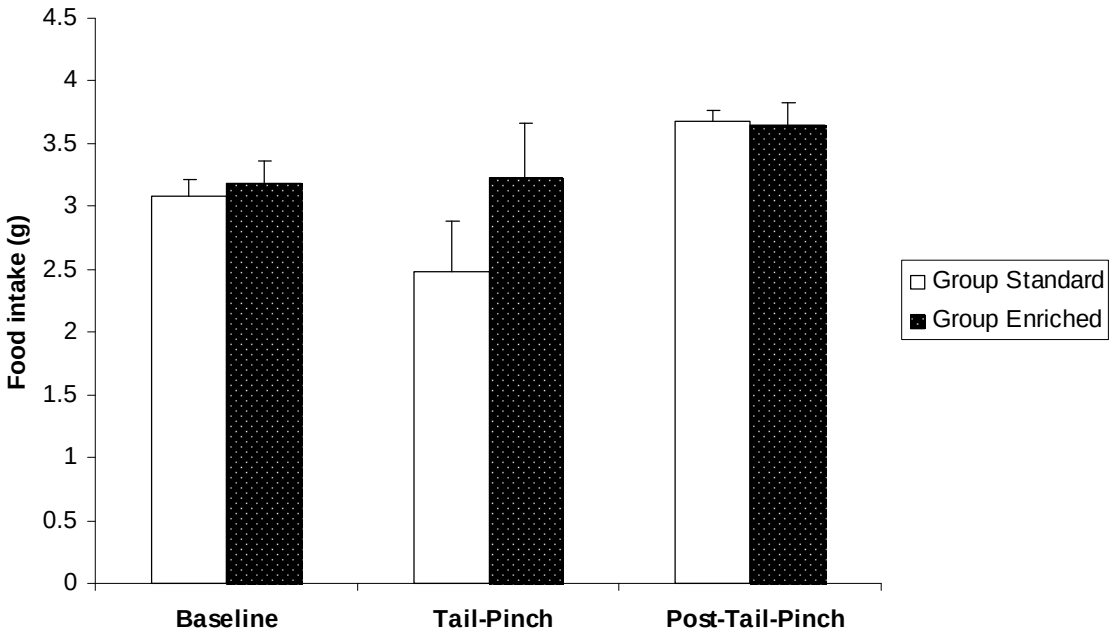

Figure 2: The Amount of Food Intake in Group Standard and Group Enriched during baseline, tail-pinch, and post-tail-pinch phases

Analysis of Individual Variability in Food Intake

Given that no systematic effect of tail pinch on food intake compared to that in the baseline phase was evident at the group level, we conducted an analysis of individual-subject data to determine whether the lack of a systematic effect in Figure 2 was representative of individual data. For each rat, *z* scores were calculated by subtracting the amount of food intake in tail-pinch session from the average food intake of the last five baseline sessions, and then dividing by the standard deviation in the last five baseline sessions. As shown in Figure 3, rats in both Group Standard and Group Enriched displayed very different responses in the tail-pinch session, with

some showing increased food intake relative to baseline, some decreased food intake relative to baseline, and some had unchanged food intake. The analysis showed that Group Standard had an average *z*-score of -1.76 (range: -13.56 to 6.71), and that the average *z*-score for Group Enriched was 1.03 (range: -8.37 to 6.67). If we define a disturbance in food intake as consumption during the tail-pinch session that was more than one standard deviation from baseline consumption (i.e., absolute value of the *z*-score >1), five out of eight rats in Group Standard and seven out of eight rats in Group Enriched showed disturbances in food intake (75% of all rats). The average absolute *z*-score for Group Standard was 4.18 (range: 0.86 to 13.56), and the corresponding average for Group Enriched was 3.12 (range: 0.84 to 8.37). This shows that food consumption generally changed during the acute tail-pinch session, relative to baseline consumption. However, the direction of the changes in food intake was not systematic: Tail-pinch had highly variable effects on food consumption across rats, which explains why Figure 2 failed to show differences in average group food intake between baseline and acute tail-pinch sessions.

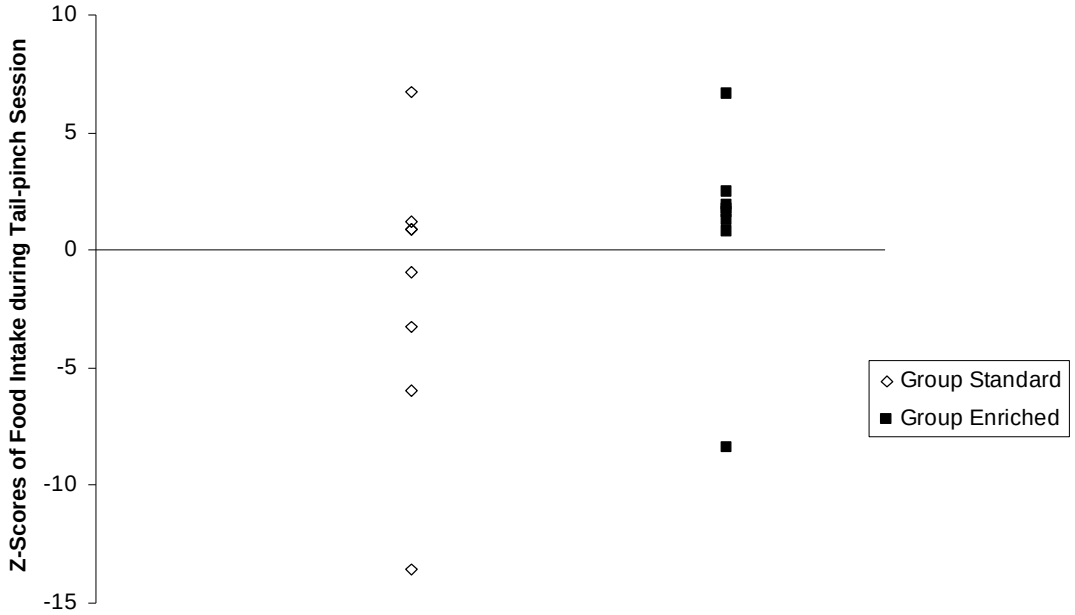

Figure 3: Z-scores of the amount of food intake in the rats during the TP phase

2.2.1.2 Body Weight

Average Body Weight in Baseline

Figure 4 shows the average post-session body weight for Group Standard and Group Enriched in the baseline sessions, with the right-most data points representing the average body weight of the last five baseline sessions. A repeated-measures analysis of variance (ANOVA) found a significant effect of time, $F(17,238)= 189.52$, $p<.001$, showing that the body weights increased across the baseline phase. There was no significant effect of group ($p> .5$), suggesting that the average body weight was similar for Group Standard and Group Enriched. However, there was a significant time x group interaction, $F(17,238)= 4.15$, $p<.001$. The weights appeared to be greater in the earlier sessions for Group Standard, but greater in the later sessions for Group Enriched.

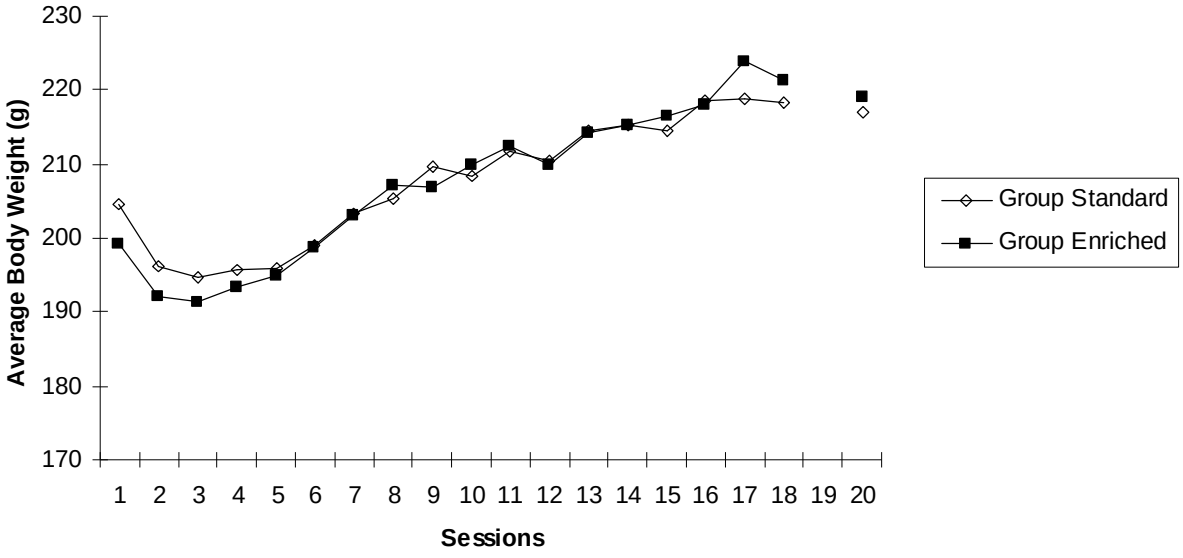

Figure 4: Average post-session body weight of Group Standard and Group Enriched during baseline and tail-pinch phases

Body Weight in Baseline and Tail-pinch Phases

Figure 5 shows the average post-session body weight of Group Standard and Group Enriched during the acute tail-pinch test. A repeated measure ANOVA found a significant effect of phase, $F(2,28)= 39.86$, $p<.001$, showing that the body weights in the post-tail-pinch phase were higher than that in the baseline and tail-pinch phases. There was no significant effect of group, and phase x group interaction (all p's > .5).

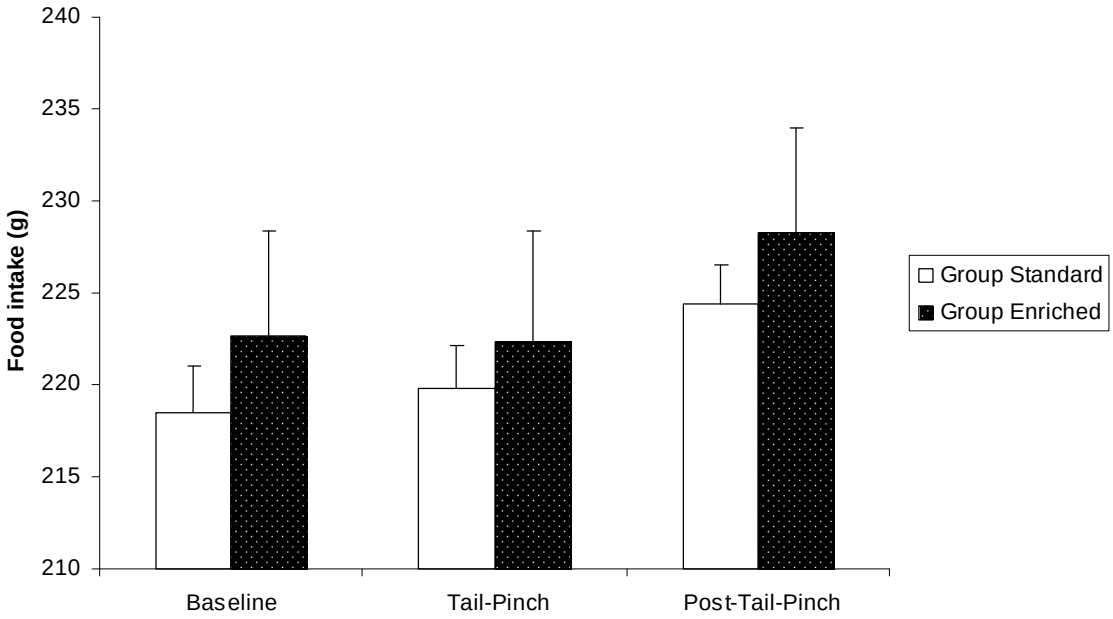

Figure 5: Average post-session body weight of Group Standard and Group Enriched during baseline, tail-pinch, and post-tail-pinch phases.

2.2.2 Chronic Tail-Pinch Test (without food deprivation)

2.2.2.1 Food Intake

Average Food Intake in Baseline

After the acute tail-pinch test session, the food deprivation protocol was discontinued and the rats were given access to free food in their home cages. Figure 6 shows the average food intake for Group Standard and Group Enriched during the baseline sessions that followed the acute tail-pinch test. The first data point represents the average baseline food intake prior to the acute tail-pinch test session. A repeated-measures ANOVA conducted on the data from baseline sessions after the acute tail-pinch test found a significant effect of group, $F(1,14)= 5.58$, $p<.05$, showing that the food intake in Group Enriched was greater than that of Group Standard. There was a significant effect of time, $F(20,280)= 9.13$, $p<.001$, showing that the rate of eating increased across the baseline phase. There was no significant group x time interaction ($p>.50$).

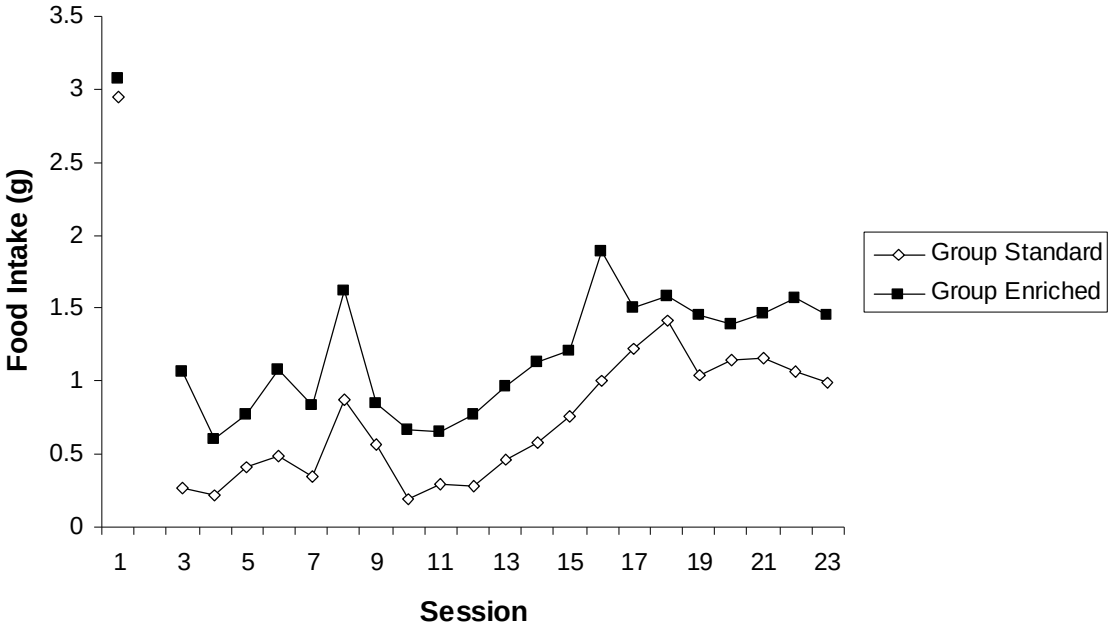

Figure 6: Average food intake of Group Standard and Group Enriched rats during baseline

Food Intake in Baseline and in Tail-pinch Phases

Figure 7 shows the amount of food intake for Group Standard and Group Enriched during the chronic tail-pinch phase, with the left-most data points representing the average food intake of the last five baseline sessions. A repeated-measures ANOVA found a significant effect of group, $F(1,13)=8.45$, $p<.05$, showing that food intake was greater overall for Group Enriched. There was a significant effect of phase, $F(9,117)=12,62$, $p<.001$, but the group x phase interaction was not significant. This confirms that food intake decreased during the chronic tail-pinch test sessions similarly for both groups.

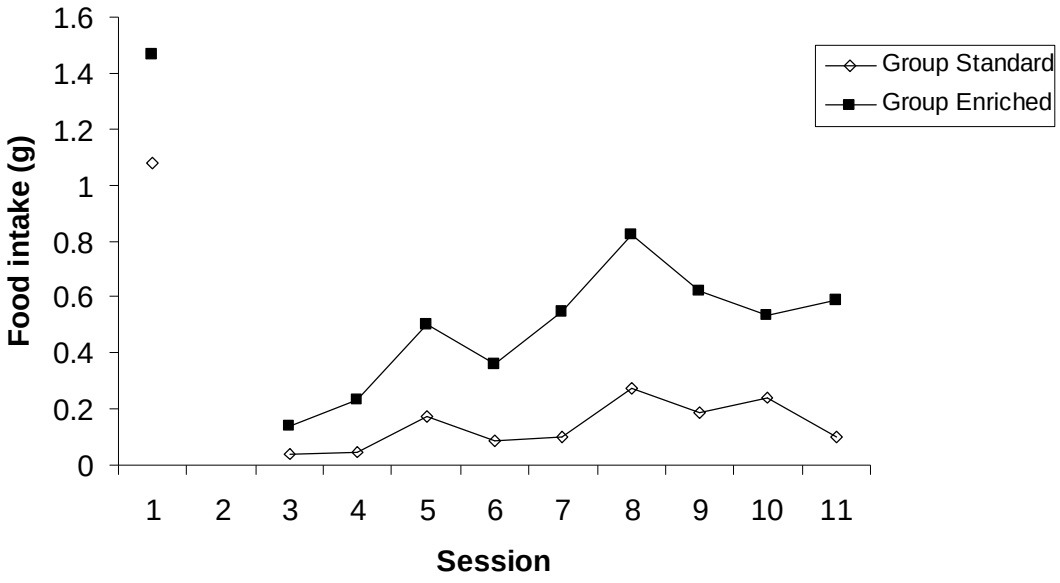

Figure 7: Amount of food intake of Group Standard and Group Enriched during the chronic tail-pinch phase and preceding baseline

Although food intake for Group Enriched was greater than that for Group Standard during the chronic tail pinch test sessions, food intake was also greater during baseline for Group Enriched. To investigate whether differences in food

intake during tail pinch were significant when differences in baseline food intake were controlled for, were conducted an analysis of covariance (ANCOVA) with baseline food intake as the covariate. Results showed that the effect of group for the three tail-pinch sessions was no longer significant ($p>.1$). This suggests that food intake during the chronic tail-pinch sessions was similar for Group Standard and Group Enriched when baseline food intake levels taken into account, consistent with the view that tail pinch affected both groups similarly. However, note that the decreased baseline food intake for Group Standard represents a differential after-effect of exposure to tail-pinch; that is, food intake during subsequent baseline sessions when rats were not food deprived was suppressed more for rats reared under standard than enriched conditions.

Food Intake in Acute Tail-Pinch and Chronic Tail-Pinch

We compared food intake across the different phases to confirm that the food-deprivation protocol had the expected effect on eating behaviour. Figure 8 shows the average food intake during the last 5 sessions of baseline prior to the acute tail-pinch test session, the average food intake during the last 5 baseline sessions prior to the chronic tail-pinch test sessions, and for the first chronic tail-pinch session. Figure 8 shows that rats ate more overall during the acute tail-pinch test and preceding baseline, when they were food deprived, than during the chronic tail-pinch test and associated baseline, when they were not food deprived. A repeated-measures ANOVA found a significant effect of phase, $F(3,42)=67.83$, $p<.01$, and a planned comparison between acute and chronic test phases (average of baseline and test session food intake) was significant. There was no significant effect of group or

phase x group interaction, suggesting that the food intake and the rate of food intake change was similar for Group Standard and Group Enriched.

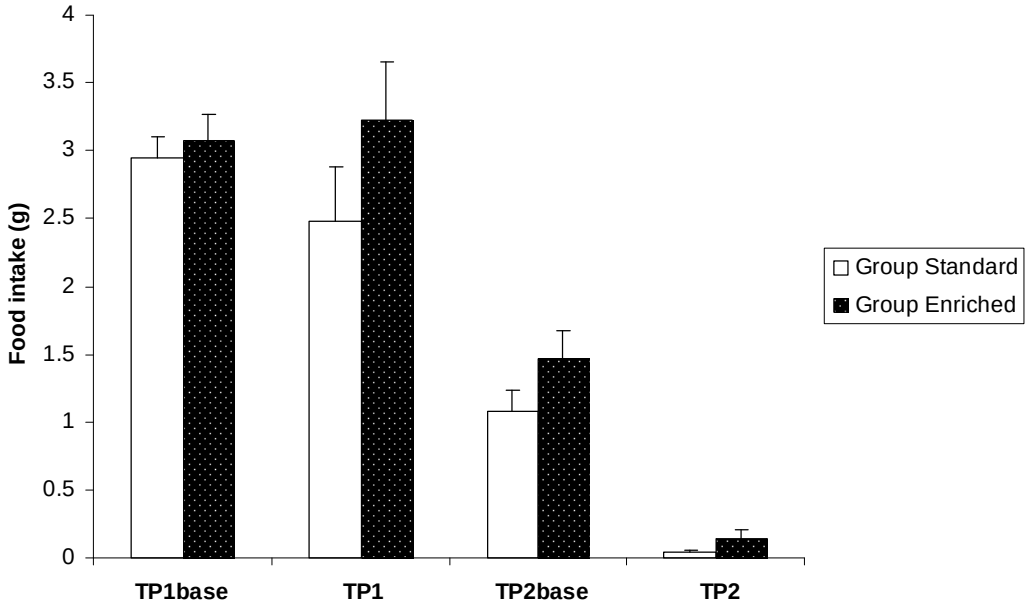

Figure 8: The amount of food intake in the acute tail-pinch test and the chronic tail-pinch test.

Average Body Weight in Baseline

Figure 9 shows the average body weight for Group Standard and Group Enriched in the baseline sessions, with the right-most data points representing the average body weight in the last five baseline sessions. A repeated-measures ANOVA found a significant effect of block, $F(20,280)= 462.95$, $p<.001$, showing that the body weights increased across sessions. There was no significant effect of group or group x block interaction ($p> .1$), suggesting that the average body weight and the rate of body weight change was similar for Group Standard and Group Enriched. Thus, body weight increased consistently for both groups after the food deprivation protocol was discontinued.

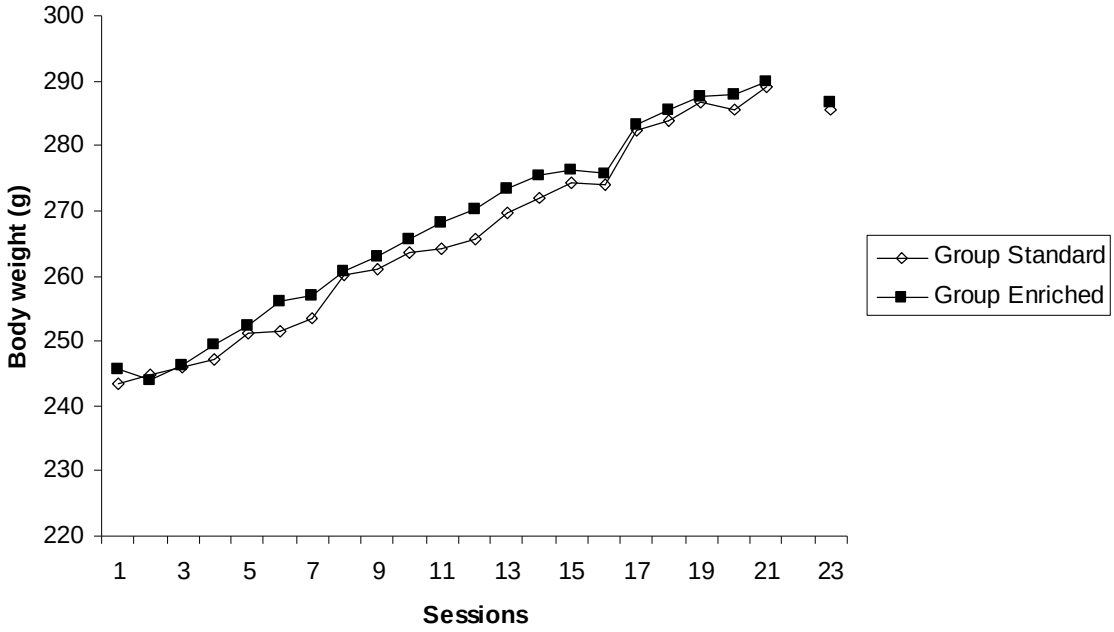

Figure 9: Average Post-Session Body Weight of the Standard and Enriched Rats during Baseline

Body Weight in Baseline and Tail-Pinch Phases

Figure 10 shows the average body weight of Group Standard and Group Enriched during the chronic tail-pinch phases, with the left-most data points representing the average body weight of the last five baseline sessions. A repeated-measures ANOVA found a significant effect of phase, $F(20,280)= 462.95$, $p<.001$, confirming that body weight increased across the tail-pinch sessions. There was no significant effect of group, and phase x group interaction (both p's > .5). This suggests that body weight continued to increase for both groups during the chronic tail-pinch test sessions.

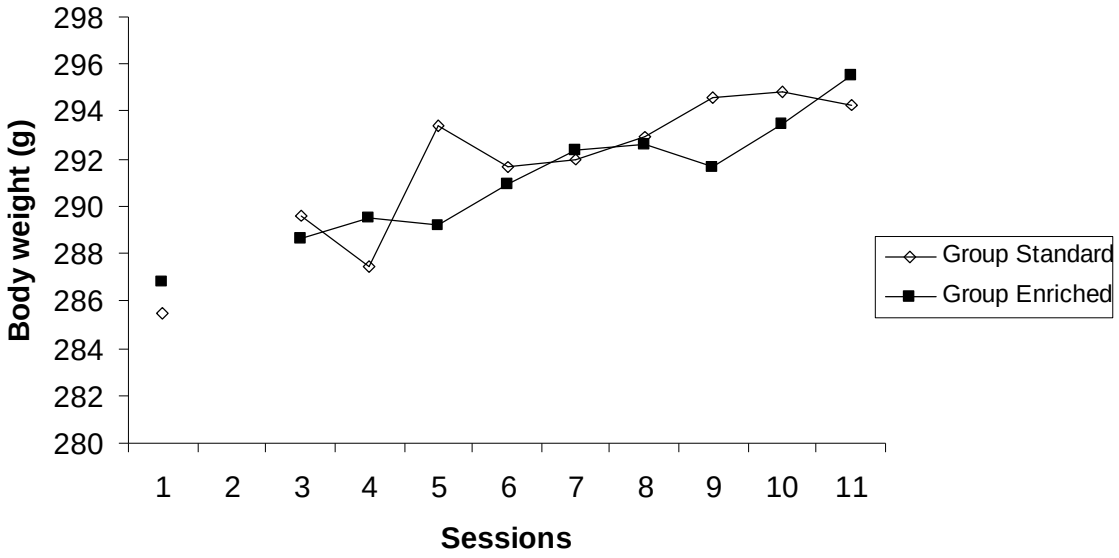

Figure 10: Group Body Weight of the Standard and Enriched Rats during Baseline and Tail-pinch Phases

2.2.3 Open Field Tests

Line Crossing

Figure 11 shows results of the open field tests that were conducted before and after the acute tail-pinch session. The figure shows the average numbers of lines crossed by the rats in the first and the second open field test for both Group Standard and Group Enriched. A repeated-measures ANOVA found a significant effect of block, $F(2,28) = 95.60$, $p < .001$, showing that the rate of line crossing decreased during both open field tests. There was no significant effect of group or test, suggesting that the number of line-crossed were the similar for Group Standard and Group Enriched, and that the rats performed similarly in both tests. Furthermore, there was no significant group x block interaction, group x test interaction, test x block

interaction, or block x test x group interaction (all *p*'s > .05). This suggests that the rate of line crossing decreased similarly for both groups across both open field tests.

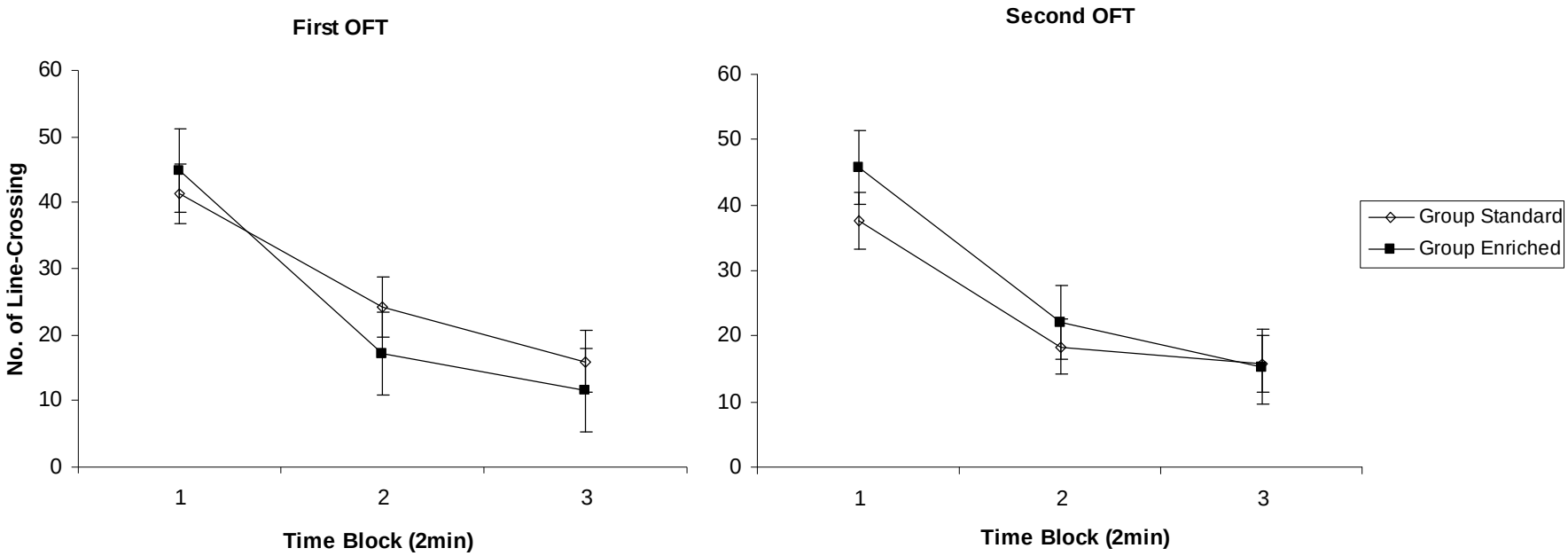

Figure 11: Number of line-crossing of Group Standard and Group Enriched in the two open field tests

Rearing

Figure 12 shows the average numbers of rearings performed by the rats in the first and the second open field test for both Group Standard and Group Enriched. A repeated-measures ANOVA found a significant effect of block, $F(2,28) = 20.69$, $p < .001$, showing that the rate of rearing decreased during the test. Although the main effects of group and test were not significant (both p's $> .05$), there was a significant group x test interaction, $F(1,14)= 5.76$, $p<.05$, showing that in the second test, the amount of rearing increased for Group Standard, and decreased for Group Enriched. There was a significant block x group interaction, $F(2,28)=4.27$, $p<.05$, showing that the rate of rearing decreased more for Group Enriched across blocks. None of the other interactions (test x block, and test x block x group) were significant (both p's $>.1$).

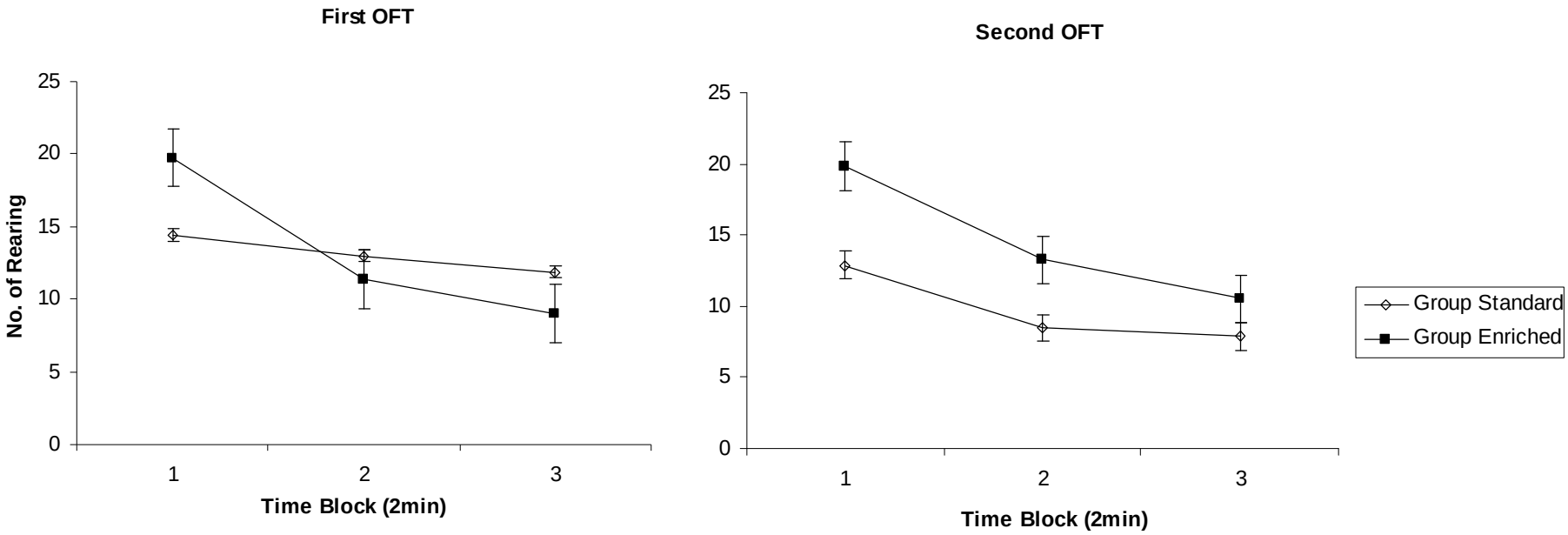

Figure 12: Average number of rearings for Group Standard and Group Enriched during the two open field tests

Latency to Novel Object

Figure 13 shows the latency to approaching the novel object by Group Standard and Group Enriched. A *t* test found a significant difference between Group Standard and Group Enriched, $t(14)=2.15$, $p<.001$, showing that Group Enriched was faster than Group Standard in approaching the novel object. However, there was a large degree of variability in Group Standard.

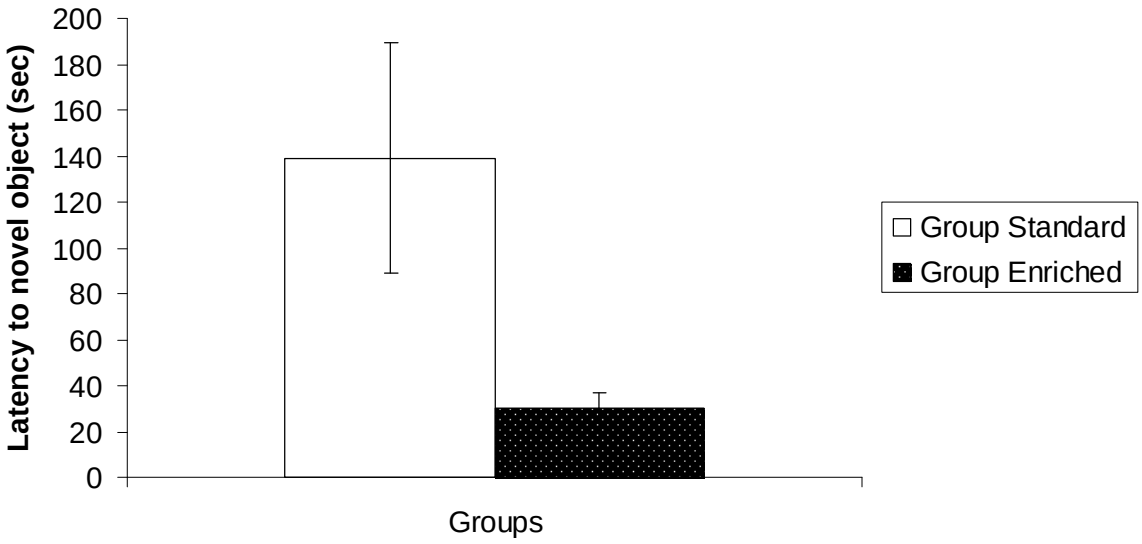

Figure 13: Average latency to approaching novel object in Group Standard and Group Enriched

Experiment 1 studied the effect of environmental enrichment on the food intake and body weight change of rats that were subjected to tail-pinch. The experiment found that before any exposure to tail-pinch, the food intake for Group Standard and Group Enriched was similar during baseline. Although the group food intakes in the acute tail-pinch session were not significantly different from those in baseline, rats showed an increase in food intake in the post-tail-pinch sessions. This

suggests that there was an after-effect of the tail-pinch on eating behaviour. Further, analysis of individual data revealed a high degree of variability in the effects of tail pinch: In both groups, some rats either increased or decreased their food intake substantially, relative to baseline, in the acute tail-pinch session.

During the subsequent baseline sessions, the rats were no longer food deprived, but they still continued to eat when placed in the experimental chamber during test sessions, suggesting that the consummatory behaviour had been conditioned to the chamber during the previous baseline training. Interestingly, it was found that the food intake for Group Standard was significantly lower than that of Group Enriched during this phase. Thus, although the groups did not differ in their eating behaviour before or during the acute tail-pinch session when they were food deprived, when they were no longer food deprived, Group Enriched showed greater food intake during baseline sessions, and consequently greater body weight as well. In the subsequent chronic tail-pinch sessions, a decrease in the food intake was observed for both groups. However, it was found that the food intake for Group Standard was significantly lower than that for Group Enriched.

The difference between Group Standard and Group Enriched during the baseline and the testing sessions of the chronic tail-pinch experiment were consistent with findings of previous research, which showed that rats reared in standard environment tended to display more anticipatory behaviours towards the stimuli than those reared in enriched environment (van der Harst, Baars & Spruijt, 2003; Piazza, Deminiere, Le Moal & Simon, 1990). In Experiment 1, the acute tail-pinch might have produced more aversive conditioning to the test chamber for Group Standard, so that it suppressed food intake more than did in Group Enriched. Furthermore, it is speculated that the tail-pinch might have also generated more anticipatory behaviours,

such as grooming and freezing, which might have competed with the eating behaviour for Group Standard.

The less suppressed food intake in Group Enriched is consistent with the findings in past studies showing that environmental enrichment can both protect against and reverse effects of stressful situations (Francis, Diorio, Plotsky & Meaney, 2002; Laviola et al., 2004). In the current experiment, having the access to enriched environment after having been exposed to the acute tail-pinch, and also between chronic tail-pinch sessions, might have further facilitated the recovery of Group Enriched, which appeared to be less affected by the stressor in terms of their food consumption. However, as expected, both groups ate less food during baseline and test sessions during the chronic tail-pinch phase when they were not food deprived, than in baseline and the test session during the acute tail-pinch phase.

Informal observations of rats during test sessions were consistent with the assumption that the tail-pinch manipulation induced an experience akin to stress in the rats. The behavioural changes included freezing, increased locomotion that was 'panic-like', increased production of faecal boli, digging of the food powder, biting of the food container, vocalisation, and tail-pinch-directed behaviours were observed. One rat in Experiment 1 even had to be dropped from the study due to its aggressive behaviours towards the experimenter after being tail-pinched. Furthermore, general differences between enriched and standard rats were noted even when rats were not subjected to tail-pinch. When being handled by experimenter, enriched rats appeared to be both more relaxed and more active in their home cages.

Overall, results from Experiment 1 suggest that tail-pinch has negative effects on rats' food intake, and that rats reared in enriched environments were more resilient to the effect of tail-pinch. Furthermore, in the two tail-pinch tests, enriched rats were

found to be more resilient to the tail-pinch effects of the acute session, as they ate more during the subsequent baseline sessions. However, the rats in Experiment 1 were subjected to tail pinch after eating behaviour had been conditioned to the testing context. This raises the question of whether differences in behaviour between rats reared in standard and enriched environments will occur when tail pinch is arranged during rats' initial exposure to the testing context. Experiment 2 was conducted to investigate this issue.

Chapter 3: Experiment 2

In Experiment 1, rats were food deprived, and the effect of tail-pinch on eating behaviour was assessed by comparison with a within-subjects baseline. The test chamber was also conditioned with eating due to the food deprivation in the baseline eating sessions. Although Experiment 1 suggested that enriched rats were more resilient to stress in the chronic tail-pinch test, there were no differences in eating when rats were food deprived, and it is unclear whether the previous exposure to the test chamber when rats were food deprived affected the results during the chronic tail-pinch test. Therefore, in Experiment 2, rats were not food deprived, and we used a between-subjects design in which rats were reared under standard or enriched conditions, and were exposed or not exposed to tail-pinch during test sessions. In this way, we planned to examine the effects of tail pinch and rearing conditions on the acquisition of eating behaviour in a novel environment.

3.1 Method

3.1.1 *Subjects*

The subjects were 20 PVG/C Male Hooded rats. They were all housed in standard cages, which were maintained in a vivarium with a 12:12 hour light/dark cycle, with lights on at 8:00 and off at 20:00, a room temperature of 21/22 degree Celsius, and the humidity at 50% during all segments of the study. After weaning, half of the rats were assigned to the standard condition, whilst the other half to the enriched condition. The rats were housed in groups of five, with food and water freely available.

3.1.2 Apparatus

Same as that for Experiment 1, except that the toys in the enriched cages were changed every day in Experiment 2.

3.1.3 Procedure

3.1.3.1 OFT

Two open-field tests were conducted for Experiment 2. The first open-field test was conducted 30 days after the rats' exposure to their assigned environments. The procedure was same as that for Experiment 1, except that the time the rat spent contacting the object was not recorded.

The second open field test was conducted three days after the first open-field test. The procedure for the test was the same as that in the first open-field test.

3.1.3.2 Eating test

Two days after the second open-field test, the rats began the consumption test, which consisted of ten daily sessions. Rats in each condition were divided into two groups, matched on body weight. One group was randomly assigned as the tail-pinch group, and the other was designated the control group. The rats were not food deprived prior to consumption sessions.

For the tail-pinch group, the procedure for the eating test was the same as that for tail-pinch sessions in Experiment 1, whereas the procedure for the control group was the same as that for baseline sessions.

3.2 Results and Discussion

3.2.1 Tail-pinch Test

Food Intake

Figure 14 shows the average food intake (g/session) for the four groups of rats in Experiment 2. A repeated-measures ANOVA found a significant effect of tail-pinch, $F(1,16)=4.62$, $p<.05$, showing that the food intake was lower in groups that received tail-pinch during the test sessions. There was a significant effect of session, $F(9,144)=3.16$, $p<.005$, indicating that food intake generally increased across sessions.

Although the main effect of environment was not significant, $F(1,16)=.07$, $p>.5$, there was a significant tail-pinch x environment interaction, $F(1,16)=4.51$, $p<.05$. This interaction occurred because tail-pinch reduced food intake in rats reared in the enriched environment (Group Enriched-NoTP > Group Enriched-TP) but not those reared in the standard environment (Group Standard-TP = Group Standard-NoTP). There was also a significant three way interaction between time x tail-pinch x environment, $F(4,64)=2.89$, $p< .05$. This interaction indicated that whereas the food intake increased at approximately the same rate over sessions for Group Standard-NoTP and Group Standard-TP, the rate of increase in food intake was greater for Group Enriched-NoTP than Group Enriched-TP. Thus, results from Experiment 2 suggest that tail-pinch suppressed the food intake of rats reared under enriched but not standard environmental conditions.

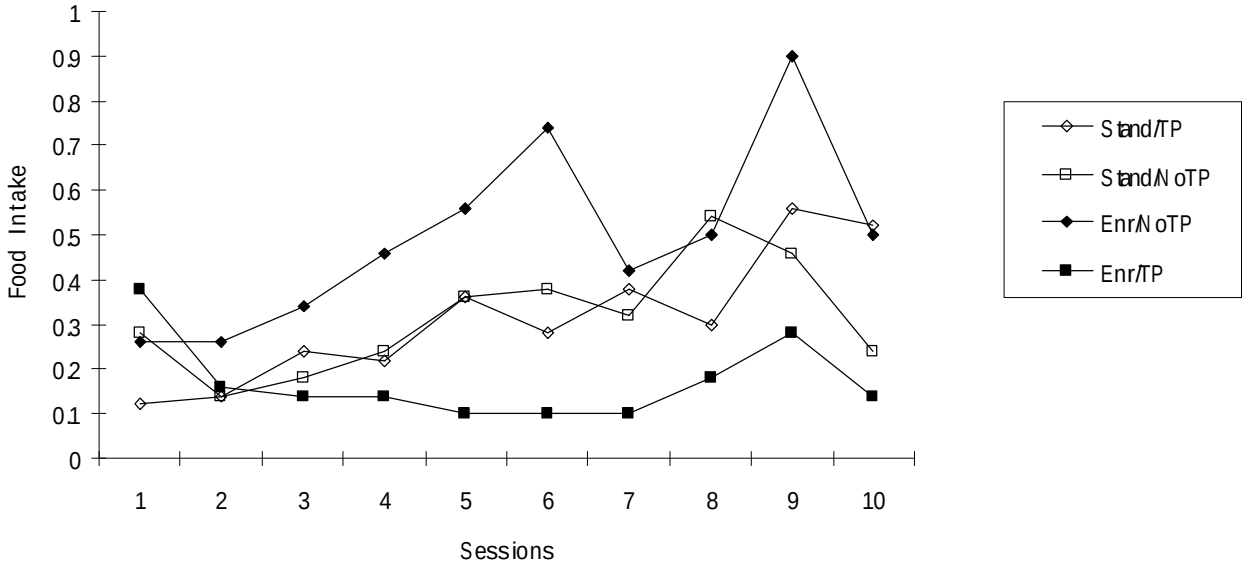

Figure 14: Average food intake for the four groups of rats in Experiment 2

Average Group Body Weight

Figure 15 shows the average post-session body weight for the four groups of rats across sessions, with the left-most data point representing the pre-session body weight for the first eating session. A repeated-measures ANOVA found a significant effect of session, $F(9,144)=79.78$, $p<.001$, confirming that the body weight increased across sessions. Although there were no significant main effects of tail-pinch or environment (both p's >.1), there was a significant time x environment interaction, $F(4,64)=8.66$, $p<.001$. This interaction showed that the rate of body weight increase was similar for the two enriched groups, but the rate of increase for Group Standard-NoTP was greater than Group Standard-TP. Thus, tail pinch suppressed the increase in body weight across sessions for rats raised in standard environments but not those raised in enriched environments. There was a significant time x tail-pinch interaction, $F(4,64)=11.32$, $p<.001$, showing that those that were subject to

tail-pinch showed lower rate of body weight gain across sessions. There was a significant time x tail-pinch x environment interaction, $F(4,64)=2.55$, $p<.05$, showing that body weight was increased for all groups but the Group Standard-TP, thus suggesting that the effect of tail-pinch was selective.

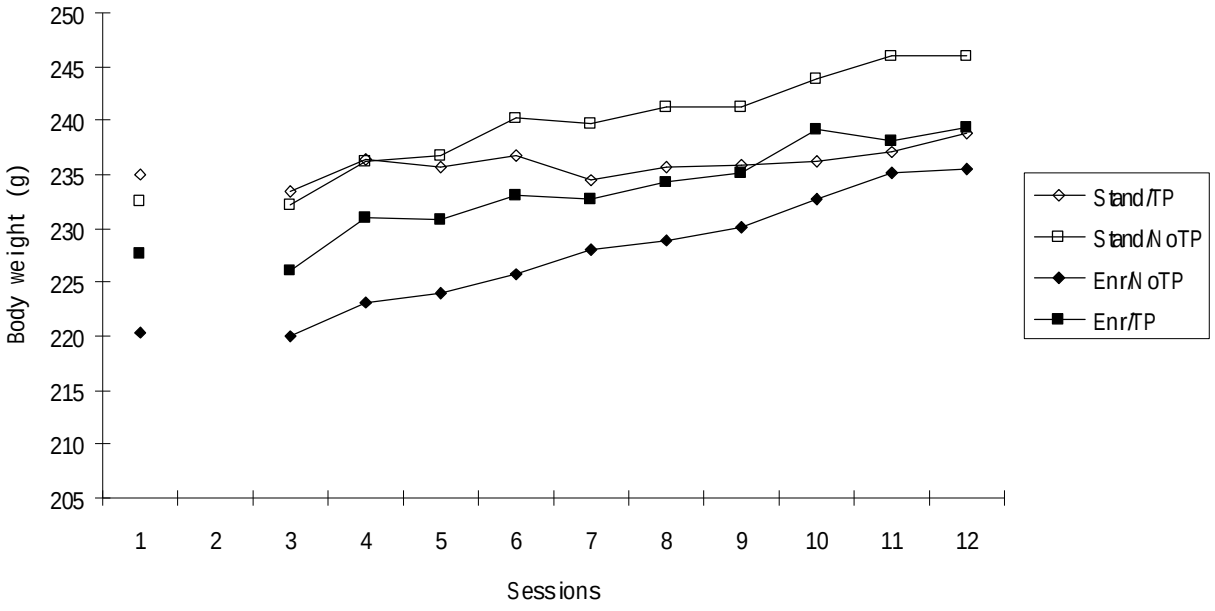

Figure 15: Average post-session Body Weight of the Standard and Enriched Rats during Testing Period

Results in Figure 15 show that body weight generally increased across sessions. Nevertheless, individual differences in rats' body weights remained very stable throughout testing in Experiment 2; the correlation between body weight prior to the first test session and the average body weight across the 10 test sessions was $r=0.96$, $p<.05$. This suggests that a more sensitive test of environment and tail-pinch might be obtained by using the pre-test weight as a covariate. Thus, we conducted an ANCOVA to examine the body weight change patterns of the groups, controlling for baseline body weight as a covariate. Prior to running the ANCOVA, the covariate

was centred by subtracting the covariate mean from each of the body weight values (Delaney & Maxwell, 1981).

Figure 16 shows body weight in each session after adjustment by baseline body weight in the ANCOVA. There was a significant effect of environment, $F(1,15)=7.60$, $p<.05$, showing that adjusted body weight was greater in the enriched groups. There was a significant effect of tail-pinch, $F(1,15)=22.31$, $p<.001$, indicating that adjusted body weight was reduced in rats that received tail pinch compared to those that did not, and a significant effect of session, $F(9,135)=82.32$, $p<.001$, showing that adjusted body weight increased across sessions. There was a significant environment x tail-pinch interaction, $F(1,15)=8.57$, $p<.05$, showing that tail-pinch significantly reduced adjusted body weight in the standard groups but not in the enriched groups. There was a significant session x environment interaction, $F(9,135)=6.84$, $p<.001$, showing that the rate of increase in adjusted body weight across sessions was greater for the enriched groups. There was a significant tail-pinch x session interaction, $F(9,135)=9.06$, $p<.001$, showing that the rate of increase in adjusted body weights across sessions was greater for control groups than for tail-pinch groups. Finally, there was a significant three-way (environment x tail-pinch x session) interaction, $F(9,135)=2.35$, $p<.05$. Closer inspection of this interaction revealed different effects of tail-pinch across sessions for standard and enriched groups. For rats reared in enriched environments, adjusted body weight increased across sessions regardless of whether they were tail-pinched or not. By contrast, for rats reared in standard environments, adjusted body weight increased across sessions if they were not exposed to tail pinch, but did not increase if they were tail pinched. Thus, analysis of adjusted body weight change suggested that tail pinch

suppressed increases in body weight to a greater extent for rats reared in standard environments than for rats reared in enriched environments.

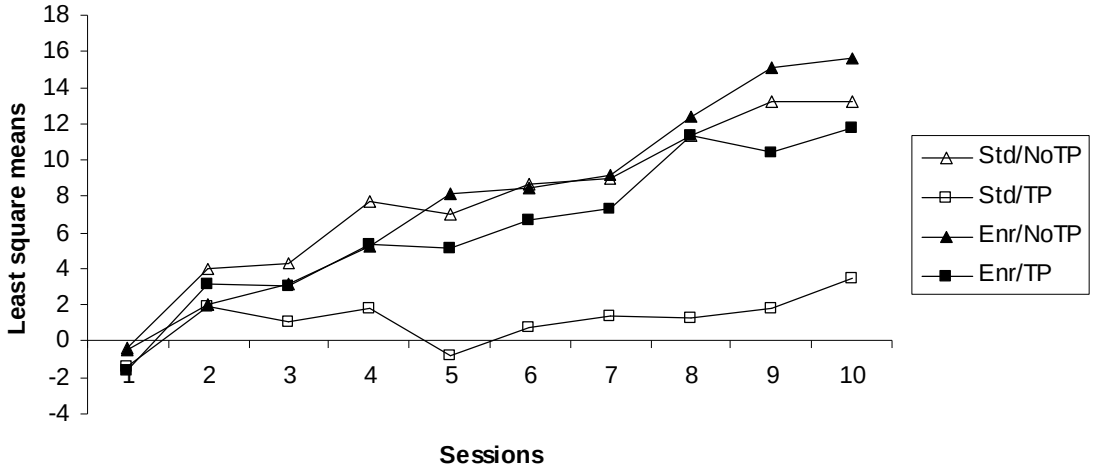

Figure 16: patterns of adjusted mean body weight (least square means) of rats in the four conditions in eating test sessions.

3.2.2 Open Field Tests

Line-crossing

Figure 17 shows the average numbers of lines crossed by Group Standard and Group Enriched in the first and the second open field test. A repeated measure ANOVA found a significant effect of group, $F(1,18)=27.91$, $p<.001$, showing that the number of line crossed was higher in Group Standard. There was a significant effect of test, $F(1,18)=17.08$, $p<.001$, showing that the number of line crossed was lower in the second open field test. There was a significant effect of block, $F(2,36)=103.55$, $p<.001$, showing that the number of line crossed decreased across blocks. There was a significant group x block interaction, $F(2,36)=4.36$, $p<.05$, showing that Group Standard showed a faster rate of decrease in line crossing. There was a significant test

x block interaction, $F(2,36)=20.13$, $p<.001$, showing that the rate of decrease in line-crossing across blocks was faster in the first open field test. Furthermore, there was a significant group x test x block interaction, $F(2,36)= 6.91$, $p<.005$, showing that rate of decrease in line crossing in the two tests was different for Group Standard and Group Enriched. For Group Standard, the rate of decrease was similar in both tests, whilst for Group Enriched, the rate of decrease was lower in the second test.

There was no significant group x test interaction ($p>.5$), showing that the Group Standard and Group Enriched displayed similar number of line crossing in the two tests.

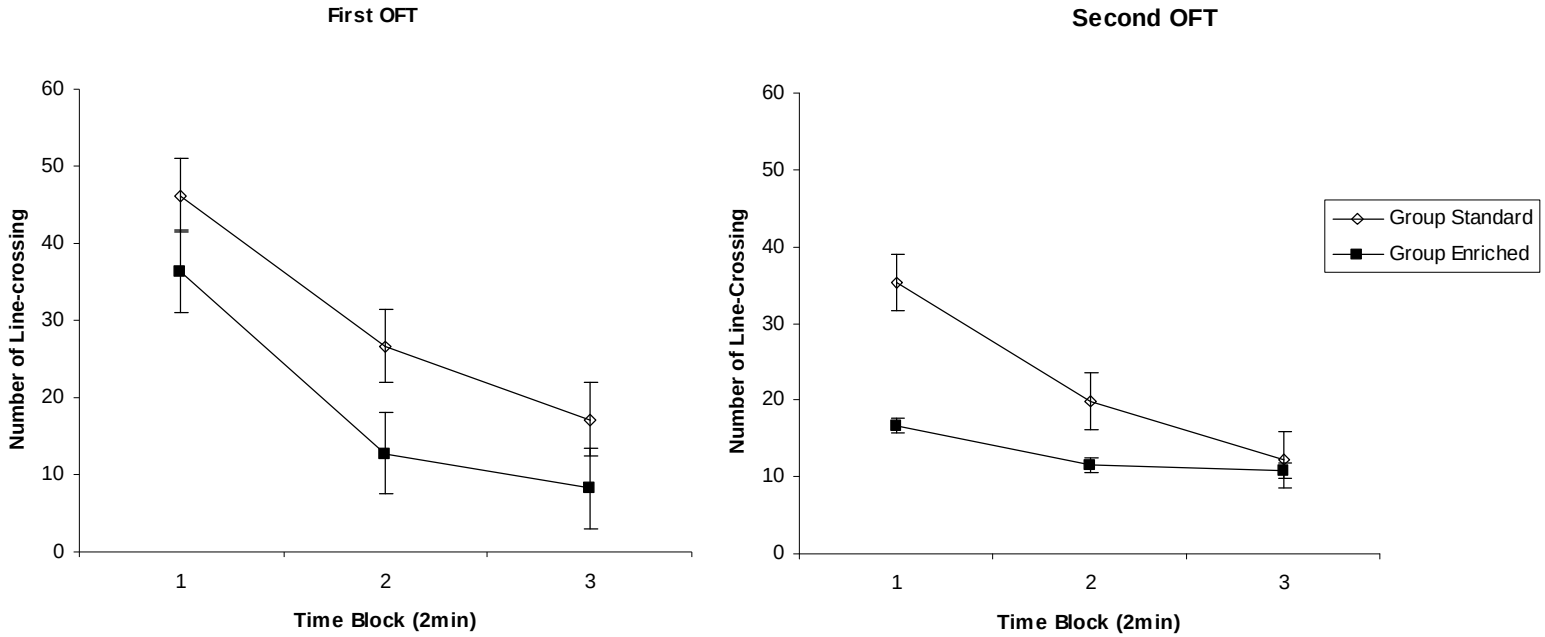

Figure 17: Number of Line-Crossing of the Standard and Enriched Rats during the First OFT

Rearing

Figure 18 shows the average numbers of rearings by Group Standard and Group Enriched in the first and the second open field test. A repeated measure ANOVA found a significant effect of group, $F(1,18)=17.9$, $p<.001$, showing that the number of rearings was higher in Group Standard. There was a significant effect of block, $F(2,36)=19.63$, $p<.001$, showing that the number of rearings decreased across blocks. There was no significant effect of test ($p>.01$), suggesting that the performance was similar in the two tests. There was a significant test x block interaction, $F(2,36)=5.76$, $p<.01$, showing that the rate of decrease in rearings across blocks was faster in the first open field test. Furthermore, there was a significant group x test x block interaction, $F(2,36)= 6.28$, $p<.01$, showing that rate of decrease in rearings in the two tests was different for Group Standard and Group Enriched. For Group Standard, the rate of decrease was higher in the second test, whilst for Group Enriched, the rate of decrease was higher in the first test. There was no significant group x test interaction, and group x block interaction (both p's$>.5$), suggesting that the group difference and the block effect was similar in both tests.

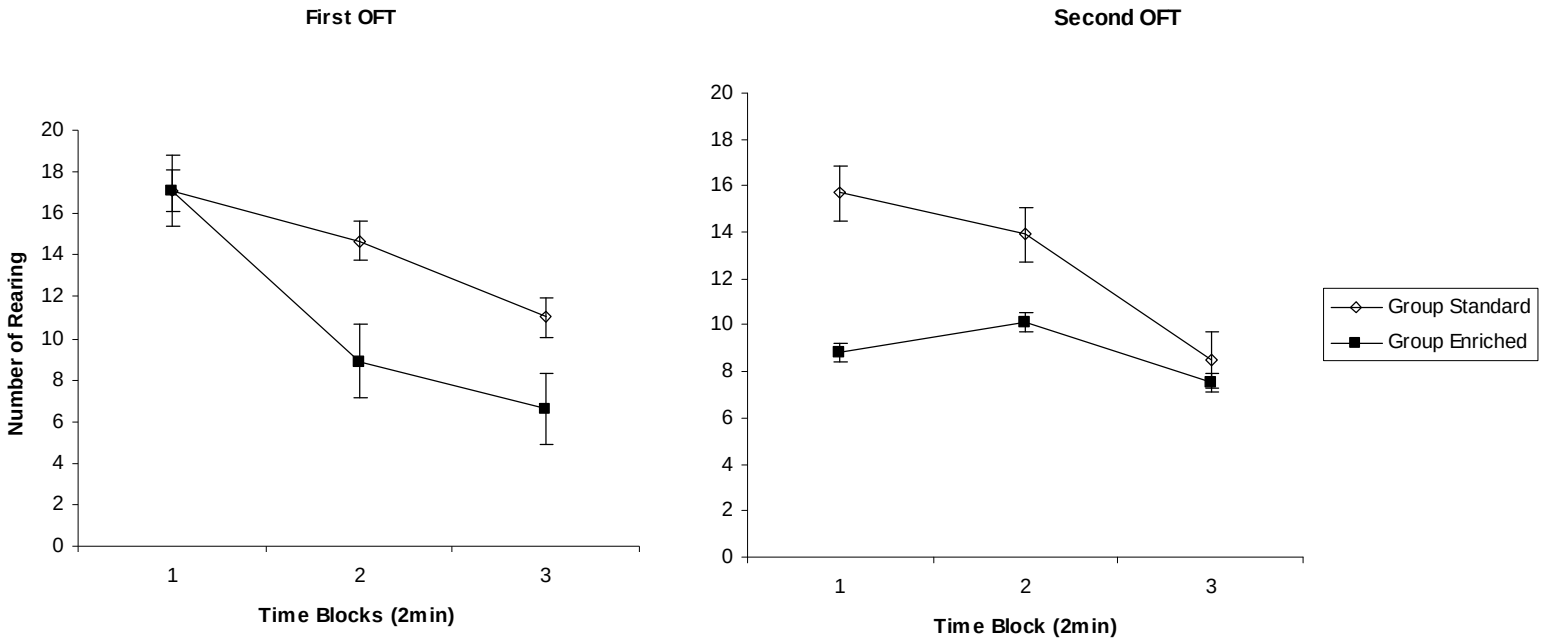

Figure 18: Number of Rearing of the Standard and Enriched Rats during the First OFT

Latency to Novel Object

Figure 19 shows the latency to approaching the novel object by Group Standard and Group Enriched. A *t*-test found no significant difference between Group Standard and Group Enriched, (p<.5), showing that the latency to approaching the novel object was similar in Group Enriched and Group Standard in approaching the novel object. However, there was huge variability for both Group Standard and Group Enriched, suggesting there were huge individual differences amongst rats in the groups.

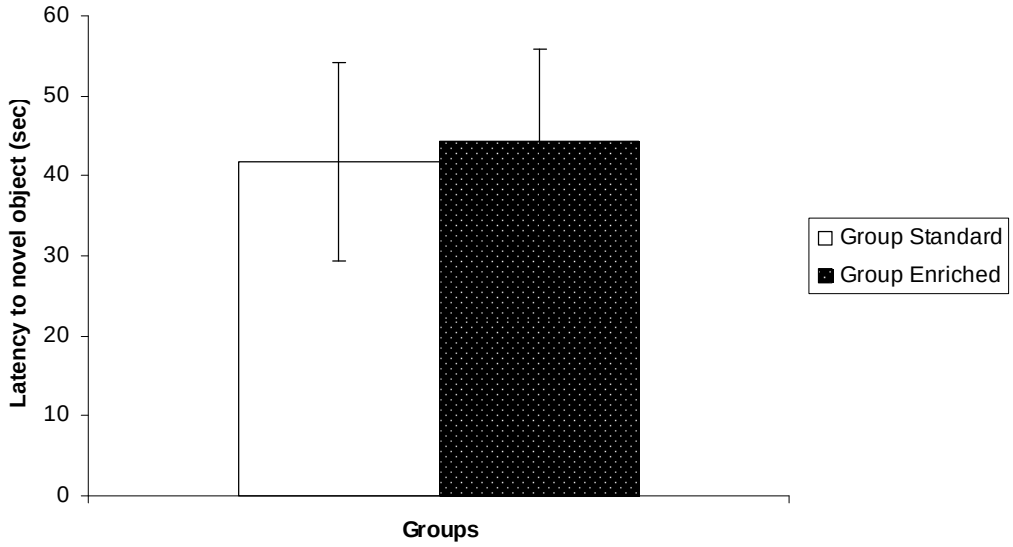

Figure 19: Latency to Novel Object in Standard and Enriched Rats

Experiment 2 showed that tail-pinch suppressed food intake during test sessions in rats reared under enriched but not standard conditions. At first this result seems surprising and paradoxical compared to Experiment 1, in which enriched rats ate more during the chronic test phase; however, examination of body weight showed that tail-pinch suppressed body weight gain in rats reared under standard but not enriched conditions. By the end of testing, body weight increase was greatest for Group

Enriched-NoTP overall and Group Enriched-TP had only slightly less body weight increase than that of the two control groups that were not exposed to tail-pinch (Group Standard-NoTP and Group Enriched-NoTP). The absence of significant difference of food intake between Groups Standard-TP and Enriched-TP implies that the tail-pinch must have had effects on the rats' eating behaviour in their home cages, with Group Enriched-TP eating more in their home cages than Group Standard-TP. The results in OFT were also consistent with that in Experiment 1, in that enriched rats showed faster rate of habituation to the open field arena. Informal observations made by the experimenter also found similar differences between enriched and standard rats, with standard rats showing more stress-related behaviours. Overall, the enriched rats were shown to be more resilient to stress. Furthermore, environmental enrichment also had protective effects against the long term effect of tail-pinch, and reduced further weight loss. A possible explanation for the apparently paradoxical findings in Experiment 1 and 2 will be described in the general discussion.

Chapter 4: General Discussion

The goal of the present research was to investigate whether environmental enrichment might mitigate the effects of stress induced by tail-pinch on rats' eating behaviour. In experiment 1 we found that rats showed a range of responses to acute tail-pinch, and that there was an increase in food intake in the post-tail-pinch sessions for all rats, indicating an after-effect of tail-pinch on consummatory behaviour. When tail-pinch was administered chronically, food intake of enriched rats were less suppressed by tail-pinch compared to that of standard rats. Apparently contradictory results were obtained in Experiment 2, in which tail-pinch suppressed the food intake of enriched rats more than that of standard rats. However, results showed that when differences in body weight prior to test were taken into account, rats reared in standard conditions had less body weight increase than rats reared in enriched conditions. These findings were consistent with findings of the OFT, which showed that enriched rats habituated more quickly to the unfamiliar environment. Thus, the findings were consistent across the two experiments: Our hypothesis that rats reared in enriched environment would show less eating changes caused by tail-pinch than those reared in standard environment was supported. This suggests that rats reared under enriched conditions are more resilient to stress.

Our results supported the assumption that rats subjected to tail-pinch experience stress-like affect, which was reflected by their changes in eating behaviours. These changes were unsystematic in terms of direction in the acute test session in Experiment 1, when rats were food deprived, but generally large in terms of relative deviation from baseline consumption. The effects of tail-pinch were systematic in

the chronic tail-pinch test in Experiment 1 and the testing sessions in Experiment 2, in which tail pinch suppressed food intake.

The experiments confirmed our assumption that rats reared in enriched environment would be different from those reared in standard environment. There was a general difference between enriched and standard rats when being handled by the experimenter, and in the open-field tests (OFTs). In the OFT in Experiment 1, enriched rats habituated to the unfamiliar situation faster, and showed less latency to approaching a novel object than the standard rats. The OFT in Experiment 2 showed a lower level of activity in enriched rats. These observations were consistent with those obtained in previous studies (Engellenner, Goodlett, Burright & Donovick, 1982; Van de Weerd et al., 2002), and suggest that enriched rats were less anxious under the stress of being placed in a novel environment. The higher level of activity observed in standard rats in the present study can be interpreted as an over-responding to novel situations due to their limited behavioural repertoire (Van der Harst, Baars, & Spruijt, 2003). The study also showed differences in the eating behaviours. Similar to the effects of tail-pinch, the effects of environmental enrichment were particularly evident in the chronic tail-pinch test in Experiment 1 and in Experiment 2 due to the longer exposure to the stressor.

The two experiments showed an apparently paradoxical finding: Enriched rats ate more during the baseline and chronic tail-pinch sessions of Experiment 1 than rats reared under standard conditions, whereas tail pinch suppressed the eating for enriched but not standard rats in Experiment 2. That is, the enriched rats appeared to be less affected by tail pinch in Experiment 1, but more affected by tail pinch in Experiment 2. However, body weight increased more for enriched rats in both experiments. When one considers the context of the test environment more closely,

the difference in the consummatory behaviour during test can be explained. In the baseline eating sessions of the chronic tail-pinch test in Experiment 1, the eating behaviour had already established through the baseline training sessions prior to the acute tail-pinch test in which the rats were food deprived. Thus, there should have been substantial appetitive conditioning to the test chamber. However, in Experiment 2, for the tail-pinched rats, the tail-pinch test consisted of repeated pairing of tail-pinch and a previously-neutral testing environment. In other words, the tail-pinched rats in Experiment 2 should have been learning an association between tail pinch and the test chamber.

Here, the two groups of enriched rats showed two very different responses, the non-tail-pinched rats showed the most food intake, and the tail-pinched rats showed the least food intake. In contrast, the two groups of standard rats did not show a significant difference, even though they were subjected to the same conditions. The difference between enriched and standard rats can be explained as a difference in the degree of contextual conditioning: The enriched rats learned an association between tail pinch and the test chamber more rapidly than the standard rats. This is consistent with the findings of Woodcock and Richardson (2000). In their study, enriched and standard rats were put in a chamber where they each received a shock with different latency (4, 6 or 120 seconds). Both groups of rats showed similarly low rate of freezing in chamber with 4-second pre-shock period, and high rate of freezing in chamber with 120-second pre-shock period, due to the lengths of time for the rats to pick up the contextual cues. However, the study found that enriched rats showed more freezing at 16-second pre-shock period than did standard rats, indicating that they were able to process contextual information more rapidly. Furthermore, enriched rats did not freeze when placed in a chamber that was similar to the testing chamber.

Similarly, past studies showed that enriched rats were better able to discriminate aversively conditioned stimuli from neutral stimuli (Barbelivien et al., 2006), and that they were less likely to generalise conditioned fear from one setting to another (Will, Galani, Kelche, & Rosenzweig, 2004). This ability to acquire the contextual conditioning faster in enriched rats can also explain the faster habituation in open field tests. It is also likely that rats in Group Standard-TP in Experiment 2 might have been more likely to generalise their fear to the home cages, which could have interfered with eating. In contrast, rats in Group Enriched-TP might be less likely to generalise their fear to the home cages, both because they process contextual information faster, and that their home cages were less similar to the test chamber. This finding suggests that in humans, those live in a more 'enriched' environment, or an environment that creates less stress, are less likely to generalise negative emotions such as fear from one context to another, thus would be more able to maintain a stable emotional state, and show less behavioural disturbances induced by negative emotions.

With the assumption that enriched rats had stronger contextual conditioning, the test chamber should be more highly valued for them in Experiment 1, and would showed more eating behaviour compared to the standard rats. However, no such difference was observed between enriched and standard rats until after the acute tail-pinch session. Nonetheless, given that all rats were food deprived in the baseline and the testing sessions in the acute tail-pinch test, there may have been a ceiling effect that masked the differences between the two groups of rats. After the acute tail-pinch session, the rats were no longer food deprived, and the difference between the two groups became more apparent.

Experiment 2 showed that rats reared in an enriched environment were better able to adapt to stress in terms of regulating their food intake in different

environments than rats reared under conditions. This finding may have implications for eating disturbances in humans, and help to explain why stressful situations bring about disturbed eating in individuals with eating disorders, even in individuals recovered from BN for 6 months (Strober, 1982), but not in individuals without eating disorders. Resilience to stress may involve being able to discriminate effectively between those contexts in which stressors occur and those in which they do not; conversely, failure to discriminate these contexts may render the individual more susceptible to the effects of stress. Further studies specifically looking at differences in contextual conditioning and discrimination between individuals with eating disturbances and healthy individuals may be able to identify the role that basic conditioning processes might play in the aetiology of eating disorders, and lead to development of more effective interventions. For example, one possibility is that the impact of stress as a trigger for binge-eating episodes might be reduced if individuals were better able to associate the stress with a specific context.

It is important to keep in mind that standard environments used in laboratories are in fact not 'standard' at all, for it has evidently served as a source of stress for laboratory animals (van de Weerd et al., 2002). Similarly, enriched environments in laboratories by no means equate the natural habitats of animals, but merely environments that are less stressful than standard environments. In the present study, we looked at the effects of two types of stressors, with the living environment as the chronic type of stressor, and tail-pinch as the acute type of stressor. The study showed that the level of chronic stress had more enduring effects on animals, and played a mediating role on how one reacted to acute stressors. Past studies also showed that environmental enrichment was able to reverse damages caused by other stressors (Escorihuela, Tobena & Fernandez-Teruel, 1994; Francis, Diorio, Plotsky & Meaney,

2002). This underlines the importance of considering chronic stressors such as overall living condition and family conflicts when providing treatments for eating disorders in humans.

Taken together, environmental enrichment had beneficial effects on rats. The findings of the two experiments in the present study are consistent, in that the established eating pattern in enriched rats was less disrupted by the one-off exposure to the tail-pinch stressor, and they were more resilient to the chronic exposure to tail pinch in both experiments. The suppression of eating during test sessions but not overall weight gain for Group Enriched-TP in Experiment 2, combined with the lack of suppression of eating during test sessions and reduction in weight gain for Group Standard-TP, also suggests that enriched rats may have learned to discriminate the contextual stimuli more effectively.

4.1 Limitations of This study and Future Directions

It is important to note a number of limitations in the present study. Firstly, the numbers of rats used in the two experiments were relatively small, which makes generalisation of the study results less reliable. Secondly, the present study used only male rats, and thus the relevance of the results for understanding the relationship between stress and eating in females may be limited. Given that eating disorders in humans are far more prevalent in females than in males, future studies using female rats will yield information that may be more applicable to the eating disorders that are generally found in females. It would be very interesting to determine whether the same relationships that were found here between environmental rearing conditions, tail pinch stress, and consummatory behaviour would also be found with female rats.

The present study was also limited in that it only looked at environmental enrichment in terms of physical stimulation. Past studies showed that social deprivation and physical deprivation affected animals differently (Elliott & Grunberg, 2005). Future research can explore the effects of both social and physical enrichment on rats' eating behaviour when subjected to tail-pinch, and also their reversal effects on disturbances caused by stress. Findings of this type of study may have important implications for the prevention and treatment of eating disturbances. The current study only looked at the effects of tail-pinch, and it will be interesting to explore the relationship between the different types of stressors and changes in eating behaviours in rats, and find out any qualitative differences between stressors. Animals have shown different eating behaviours towards different types of food in past studies (Morand-Feron, Lefebvre, Reader, Sol & Elvin, 2004). Future research can adopt the current study design and use different types of food to see whether rats react differently.

The tail-pinch applied in the present study appeared to be of a higher intensity than that used in Rowland and Antelman's (1976) study, despite our intention of replicating their tail-pinch method. In their study, Rowland and Antelman (1976) reported that their tail-pinch did not seem to distress the animals, and induced eating behaviour immediately. This is different from our observation. According to the literature, mild stress appeared to increase eating, whereas more intense stress appeared to reduce eating (Mohammad, Chowdhury, Fujioka & Nakamura, 2000). However, to our knowledge, there has not been a study systematically examining the effects of tail-pinch with different intensities on animals. The inconsistent effects of tail-pinch reported in past studies may explained by the different intensities of the tail-pinch. On the other hand, the inconsistent effects of tail-pinch could also be due to

the individual variability amongst rats towards the stressor. This was evident in the Acute Tail-Pinch Test in Experiment 1 of the present study, where rats responded differently to the tail-pinch. Future research exploring the impact of stress at different intensities, and specific roles individual variability play will hopefully provide more understanding of the effects of tail-pinch as a stress manipulation.

4.2 Conclusions

Overall, the results of the present study confirmed the beneficial effects of environmental enrichment on animals observed in past studies. The present study examined eating behaviours of rats under stress and showed that rats reared in enriched environments were more resilient to stress. Furthermore, the enriched rats appeared to be faster at acquiring contextual cues, and displayed more stable eating patterns once they were conditioned to the environment. This was consistent with past studies and shed some light on the difference between individuals with eating disorders and healthy individuals. Furthermore, the present study also found different effects of chronic and acute stressors on rats' stress-coping abilities, and thus highlighted the importance of considering the overall chronic living condition of an individual with eating disorders. Hopefully future research with will yield greater understanding of the relationship between environmental conditions, stress and eating behaviour in rats, leading to an animal model for human eating disorders which will lead to more effective treatments for those who suffer from these disorders.

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